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OBSERVATIONS ON THE CELL MASSES IN THE CEREBRAL HEMISPHERE OF THE NEW ZEALAND KIWI (*APTERYX AUSTRALIS*)[1]

By ARCHIBALD DURWARD, M.D.

Institute of Anatomy, University College, London

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INTRODUCTION

UNTIL quite recently the internal anatomy of the brain of the New Zealand kiwi has escaped the attention of comparative neurologists, although some studies of the external form and development of the brain were made in the latter part of the last century by Parker (1891). This lack of study of the kiwi's brain was due doubtless to the paucity of material available outside the Dominion of New Zealand. Hunter (1923) and Craigie (1929 and 1930) of recent years have investigated the internal anatomy of the brain, and their accounts, particularly Craigie's, have proved of great assistance in the present study. The material on which their studies were based was, however, limited to two brains, both sectioned transversely and one of which Craigie states was not of great use for any detailed study of the internal anatomy.

[1] The observations recorded in this paper are the result of work carried out by the writer while in the Department of Anatomy, University of Otago, New Zealand.

Although it might be expected that numerous kiwi brains would be readily obtainable for scientific investigation within New Zealand, this unfortunately is not so, for the bird is rigorously protected by the authorities on account of its supposed threatened extinction. To date three birds have been granted to this laboratory, and on the brains of these specimens the present study has been made.

MATERIAL AND TECHNIQUE

The birds were taken in the wild state on Stewart Island off the coast of the South Island of New Zealand and are of the species *Apteryx Australis*. To ensure the best possible fixation the birds were delivered alive at the laboratory where they were killed and the brains at once removed and suitably treated. Two specimens were prepared by the toluidin-blue method of Huber (1927) for cell staining and gave excellent results. Of these one was sectioned transversely (G.B. 81) and the other in the sagittal plane (G.B. 95). The third was prepared by the Weigert method (G.B. 92) and sectioned transversely. All the technical work was most successfully carried out by Mr G. Howard of the Anatomy Research Laboratory of the University of Otago.

I would express my gratitude to Dr E. Horne Craigie of Toronto for placing at my disposal the manuscripts of certain of his papers on the kiwi brain prior to their publication, and for his personal communication in which he expanded somewhat the views expressed in his published works. To Prof. Wallenberg of Danzig I am deeply indebted for the loan of the historic paper on the fore-brain of birds by Edinger, Wallenberg and Holmes.

It is a pleasant duty to express my gratitude to my former chief, Prof. W. P. Gowland, University of Otago, New Zealand, who first suggested this study and who has always taken a stimulating interest in its progress.

OLFACTORY BULB

The olfactory bulb of *Apteryx* is a prominent mass lying ventrally at the rostral extremity of the hemisphere, from under which it projects (figs. 1, 2 and 3). There is no fusion of the bulbs as in certain other forms, and a peduncle is entirely absent. The general form of the bulb may be understood by reference to figs. 4–8 and 26–28. It will be seen that the various layers are as it were wrapped round the rostral extremity of the hemisphere in the form of a curved plate. This accounts for the appearance of concentric lamination produced in fig. 4 of Craigie's 1930 paper. To that writer's clear account of the various layers comprising the bulb I can add no new facts, beyond that the olfactory ventricle in my series is continuous by means of a narrow canal with the main cavity of the lateral ventricle. The various layers of the bulb have been indicated in the figures.

BASAL CENTRES OF THE LATERAL FORE-BRAIN WALL

Palaeostriatum

Lying ventro-medially in the hemisphere this mass forms as it were the core of the striatum complex whose other components are superimposed on it in a laminated manner. Its extent and boundaries are readily recognisable in both cell and fibre preparations. The lamina medullaris dorsalis forms a prominent boundary line dorsally and laterally throughout its whole extent. In transverse series the palaeostriatum generally forms a somewhat hemispherical mass (figs. 11–18), its contour altering, however, at the rostral and caudal extremities. Figs. 26–29 show the appearance in sagittal sections where it forms rather an L-shaped mass with its angle projecting forwards and dorsally and giving the appearance of having pushed the other masses in the hemisphere in front of it.

Fig. 1.

Within the palaeostriatum a subdivision may be made into palaeostriatum augmentatum (mesostriatum of some authors) and palaeostriatum primitivum (nucleus entopeduncularis of Edinger, Wallenberg and Holmes, 1903). This terminology, which follows that of Huber and Crosby (1929) and is in agreement with the usage of Ariëns Kappers in his 1928 and 1929 publications, differs from that of Craigie (1930) in his study of *Apteryx*. The latter writer, however, has correlated his terminology with the various fields of Rose (1914). Craigie's mesostriatum in *Apteryx* corresponds with field *H* of Rose and with my palaeostriatum augmentatum; Craigie's palaeostriatum in *Apteryx* corresponds with field *J* of Rose and with my palaeostriatum primitivum. Of these

two components the palaeostriatum augmentatum is considerably the more extensive; the palaeostriatum primitivum is in *Apteryx* but feebly represented as compared with the size of the similar mass in certain forms described by Huber and Crosby (1929).

1. The palaeostriatum augmentatum is shown at its maximum development in figs. 12–14. Dorsally and laterally it is related to the neostriatum and ectostriatum, but separated from them by the well-defined lamina medul-

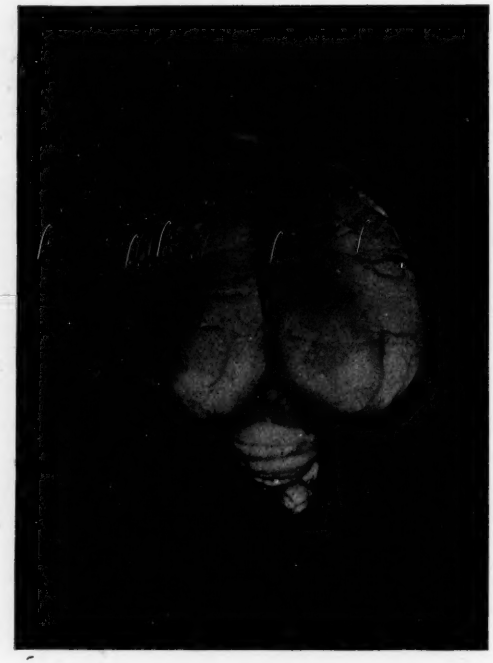

Fig. 2.

laris dorsalis. Ventrally the mass abuts on the surface of the hemisphere, but is in part separated from the pia by the intervention of the tractus olfactohabenularis. The ventral and lateral limit of the palaeostriatum is in many sections (figs. 11–16) rendered very evident by the presence of a well-marked fissure, fissura ventralis, which indents the brain wall at the ventral limit of the lamina medullaris dorsalis. Blood vessels may be seen entering at this fissure and passing deeply into the brain along the line of the lamina medullaris dorsalis.

Dorsally and medially the palaeostriatum augmentatum reaches the ventricle and at certain levels (figs. 15–20) a ventricular depression is evident

overlying the mass. A faint fissura neo-palaeostriatica may be detected in figs. 17 and 20, but is certainly not a constant feature at all levels.

Rostral to the level represented by fig. 10 the palaeostriatum augmentatum gradually decreases in size, loses contact with the ependyma, and becomes related ventrally to the area praepyriformis. At its rostral extremity (fig. 9) the palaeostriatum augmentatum is represented merely by an isolated mass of cells in the midst of the neostriatum, just caudal to the level of the bulbar formation.

Fig. 3.

Caudal to the level of fig. 18 the contour of the palaeostriatum augmentatum is affected by the presence and gradual increase in size of the archistriatum which lies ventro-medial to it and gives it a comma shape (fig. 20). Further caudally still the palaeostriatum augmentatum comes to lie almost directly dorsal to the archistriatum, but does not extend so far caudally as it. Fig. 21 shows the arrangement clearly in this caudal part of the palaeostriatum. The relationship of the caudal extremity of the palaeostriatum augmentatum to the neostriatum caudale is illustrated in fig. 22.

The cells comprising the palaeostriatum augmentatum are mainly small and take a light stain with toluidin blue. Intermingled with these small cells are some definitely larger cells. The distribution of cells is generally uniform and not very dense. Scattered throughout the mass at all levels are small

aggregations of cells which take a somewhat denser stain than the other cells. These aggregations are neither large nor numerous but are so constantly present that they lend a characteristic appearance to the palaeostriatum augmentatum. The radiating bundles of fibres of the lateral fore-brain bundle give a somewhat radiating arrangement to the cells in the centre of the mass.

2. The palaeostriatum primitivum (i.e. palaeostriatum of Craigie, 1930) is not by any means a well-defined nucleus in *Apteryx*. Its presence in association with the lateral fore-brain bundle is undoubted, but in the description and figures of Huber and Crosby (1929) it is evident that in other forms this mass reaches much more considerable proportions. The characteristic features it possesses in other forms is apparently the large size of its cells. In *Apteryx* cell size is certainly slightly greater in palaeostriatum primitivum than in the palaeostriatum augmentatum, but the difference is not great. The position of the mass is indicated in figs. 15–19, but it must be clearly understood that the mass is of small proportions and its cells neither numerous nor strikingly large. Its rostro-caudal extent is considerably less than that of palaeostriatum augmentatum.

Archistriatum

The archistriatum or amygdaloid complex commences rostrally at about the level of the foramen of Monro and extends backwards therefrom. It lies ventral and medial to the caudal end of the palaeostriatum, in which it lies partially embedded (fig. 20). Within the archistriatum there would appear to be several components, a suggestion in favour of which Craigie (1930) has already found evidence.

The largest component which, according to Craigie's usage, may be called pars magnocellularis archistriati, is very definitely associated with the downward-sweeping bundles of the commissura archistriatica. This mass is on the whole of a spherical shape and lies with its rostral extremity embedded in a depression in the caudal part of the palaeostriatum (figs. 21 and 30). The cells are discrete and occasionally arranged somewhat radially in accordance with their association with the fibres of the commissura archistriatica. Their size is distinctively large and the staining with toluidin blue fairly dense. Huber and Crosby (1929) have noted how the large-celled component of the archistriatum may be further divided into dorsal and ventral parts due to difference in cell size. This feature is evident in my preparations of *Apteryx*; the dorsal and caudal part of the pars magnocellularis contains cells which are larger than those in the ventral part. There is, however, no exact line of demarcation between these two parts of the pars magnocellularis for they fade imperceptibly into each other. At the caudal extremity of the archistriatum and abutting on the ventricle is a small circumscribed mass which is characterised by the close packing and dense staining of its cells. Figs. 29 and 30 show this mass (not labelled) at the caudal extremity of the archistriatum. Transverse sections did not allow of its identification so readily as did the sagittal. It

may quite possibly be a separate subdivision of the archistriatum, but is included here with the pars magnocellularis with which it lies in closest contact.

Between the pars magnocellularis and the palaeostriatum there is in cell preparations a cell-free zone which is seen in fibre preparations to be occupied by a lamina of medullated fibres. Indeed the whole pars magnocellularis forms in Weigert preparations a readily recognisable area owing to the very considerable preponderance of medullated fibres which permeate it.

The other components of the archistriatum are smaller and much less conspicuous than the main part described above. Craigie (1930) has described a pars parvocellularis which he considers to form a medial subdivision of the archistriatum. With this I agree and also that this pars parvocellularis corresponds with the nucleus taeniae as described by Edinger, Wallenberg and Holmes (1903), Schroeder (1912) and Huber and Crosby (1929). The pars parvocellularis lies in general medial to the pars magnocellularis. Rostrally it is insinuated between the tractus olfacto-habenularis and the archistriatal commissure (fig. 18), whilst caudally it shifts in a ventral and lateral direction intervening here between the pars magnocellularis and the brain surface (fig. 20). The mass is less easy to define caudally because here it becomes a very thin lamina of cells and spreads out ventrally just under the pia. The cells comprising the pars parvocellularis are mostly small and faintly staining. There are, however, intermingled with these small cells some definitely larger ones. Its demarcation from the pars magnocellularis is readily seen owing to a relatively cell-free zone intervening between them. In Weigert preparations it is evident that the pars parvocellularis has not the same intimate connection with the commissura archistriatica as has the pars magnocellularis.

The third component of the archistriatum is the smallest and its identity as part of the archistriatum is only tentatively put forward. Both transverse and sagittal series in the toluidin-blue preparations show this mass, but my Weigert preparations lend no aid as to its connections. That it is a separate mass from either magnocellular or parvocellular portions of the archistriatum I have no doubt, and its inclusion along with the archistriatum is made because of its proximity to that mass and the inability to find, as yet, any reason for including it with another cell group. I have named this group of cells the pars ventralis archistriati from its position relative to the other archistriatal components. Figs. 18–20 show it in transverse series where it appears as a circular group of cells lying ventrally in the archistriatum. A comparison of these figures clearly shows that the pars ventralis is gradually becoming more ventrally placed when traced caudally. Fig. 30 shows the same mass in sagittal section, where it appears as an oblique column of cells lying ventral and somewhat rostral to the main archistriatal mass. Its distinction from magnocellular and parvocellular components is made clear by cell-free zones which separate it from them and also by the nature of its cells which, although varying in size, have amongst them elements almost as large as any in the pars magnocellularis, but not so deeply staining. It does not

coincide in rostro-caudal extent with either of the other two components of the archistriatum, for it extends further rostrally than the pars magnocellularis and falls short of both caudally.

In the relatively cell-free zone between pars magnocellularis and pars ventralis are some small faintly staining cells which would appear to be continuous with the pars parvocellularis medially and possibly with the palaeostriatum laterally.

Craigie (1930) describes and figures an amygdaloid fissure which indents the medial aspect of the hemisphere wall, and serves to indicate the ventral border of his pars parvocellularis archistriati. He then proceeds to explain the various features of the avian archistriatum, using as his basis the work of J. B. Johnston (1923). I fail to detect with any degree of certainty such a fissure as Craigie describes. Certainly there are indentations on the medial wall in the region of the archistriatum and the brain wall here, moreover, is perforated in various places by blood vessels, but I can see no regular depression suitably situated to warrant my agreement with Craigie in this matter.

Neostriatum

The neostriatum of this description corresponds with the area similarly named by Ariëns Kappers in his 1928 and 1929 publications, and by Huber and Crosby in their study of the avian striatum (1929). Craigie in both his 1928 and 1930 papers subdivides the neostriatum somewhat differently from the above writers. In their paper Huber and Crosby have discussed at length the question of Craigie's usage in this connection, and I need state here only that the neostriatum of this description corresponds with Craigie's neostriatum inferius, as he used that term for both humming bird (1928) and *Apteryx* (1930).

The delimitation of the neostriatum from adjacent striatal components is not difficult, for medially the lamina medullaris dorsalis is readily picked up, and the lamina hyperstriatica, or the relatively cell-free zone occupied by it, gives the boundary line laterally and dorsally (e.g. figs. 13–17). The general appearance given in these figures persists throughout the major part of the hemisphere; only caudally and rostrally does any difficulty arise in the interpretation of the neostriatum, for in these regions there is some difficulty in determining the limits of the mass and in unravelling its subdivisions. One may state at the outset that the subdivisions identified by Rose (1914) (fields G, G_1 and G_2), and the corresponding zones of Huber and Crosby (1929) (neostriatum intermediale, neostriatum frontale and neostriatum caudale), although collectively corresponding to my neostriatum are not individually identifiable in all cases in my preparations.

The neostriatum throughout its major extent is a somewhat C-shaped mass abutting on the ventricle medially and dorsally, and reaching the brain surface ventrally (figs. 10–22). Ventricular fissures may at times be detected, indicating its separation from the subjacent palaeostriatum (fissura neo-palaeostriatica) and the overlying hyperstriatum (fissura neo-hyperstriatica); but in general

such fissures are not well marked, particularly the latter. At its ventral extremity the neostriatum produces a bulge of the brain wall immediately lateral to the fissura ventralis.

The cells comprising it are intermediate in size between those of the palaeostriatum and the hyperstriatum. They are larger, slightly more closely packed and take a denser stain with toluidin blue than the palaeostriatal cells. Cell size, however, is not quite uniform; intermingled with the larger distinctive cells is quite a fair proportion of much smaller cells. Adjacent to the ectostriatum there is a lamina in the neostriatum in which the larger elements are aggregated. At this point too the ectostriatum is not sharply marked off from the neostriatum by any cell-free or fibre zone; but this aggregation medially in the neostriatum of cells which are distinctively larger than the ectostriatal cells serves adequately to distinguish the two masses. The passage of fibres through the neostriatum gives it, particularly in its lateral part, a distinct radially striated appearance.

When traced rostrally the neostriatum increases at the expense of the palaeostriatum and its ventral extremity widens out, sweeping up laterally over the most ventral portion of the hyperstriatum (fig. 9). The area praepyriformis is now also in evidence lying ventrally and ventro-medially to the neostriatum. The most rostral part of the neostriatum is arranged in a somewhat complex manner as may be judged from the appearances shown in figs. 8–4 of transverse sections, and figs. 26 and 27 of sagittal sections. After sweeping ventrally in front of the palaeostriatum, the neostriatum then arches round the ventral aspect of the hyperstriatum and so comes to lie rostral to it. The appearances shown in figs. 5, 6, 8 and 9 of the transverse series are the results of sections at about the levels indicated in fig. 26 (see also legend to fig. 26). That portion of the neostriatum lying rostral to the hyperstriatum is assumed by Craigie (1930) to be part of the hyperstriatum accessorium, and although he could not detect any boundary line between this part of the hyperstriatum accessorium and the neostriatum in his material, which was cut transversely, he suggested that such a distinction might possibly be evident in sagittal series. I find that neither transverse nor sagittal series of cell or fibre preparations allow of any distinction being drawn between the two zones under discussion. The lamina hyperstriatica sweeps round in unbroken continuity, extending ultimately to the brain surface (fig. 27). The hyperstriatum accessorium, as it overlies the anterior part of the hyperstriatum is reduced to exceedingly meagre proportions, but even so I cannot detect any continuity whatsoever between it and the most rostral part of the neostriatum. This feature will be referred to again when the hyperstriatum accessorium is discussed.

Caudally from the level represented in fig. 19 the neostriatum retains virtually the same arrangement for some distance; then gradually it spreads laterally to intervene between the hyperstriatum and the pia. At the same time the hyperstriatum gradually moves dorsally and its line of separation

from the neostriatum becomes, instead of a C-shaped lamina seen in fig. 19, almost a straight line running transversely outwards from the ventricle. In this way the neostriatum towards its caudal extremity becomes relatively bulky as compared with the other structures in the section (figs. 22 and 23).

Certain specialisations also may be detected in the neostriatum in its most caudal part. Craigie (1930) likewise found some evidence of such features and he states that the specialised zone which he found caudally in his neostriatum inferius was "probably the field G_2 of Rose or neostriatum caudale of Huber and Crosby, but certainty as to this homology could not be attained." Like Craigie I am quite unable to detect any suggestion of a division into neostriatum frontale and neostriatum intermediale, but certainly at the caudal extremity of the striatum there are features suggestive of the neostriatum caudale.

As the palaeostriatum decreases towards its caudal extremity some cells lying medial to it, between it and the archistriatum, would appear to form separate nuclear masses. Fig. 22 shows the zone in question and a comparison with Craigie's fig. 6 *g* shows that the mass we refer to is the same. The first portion of the neostriatum in this caudal zone lies directly medial to the caudal extremity of the palaeostriatum. Dorsal to it in fig. 22 is a transversely placed zone of closely packed small cells, stretching between the palaeostriatum and the ventricle, and separating it from the most dorsal portion of the neostriatum. This small-celled zone might perhaps be regarded as a specialisation of the periventricular grey matter. It is apparently the same zone which Craigie has labelled "*L*" in his fig. 6. The other mass just ventral to this small-celled zone I consider to be something more than merely the extension of the neostriatum between the caudal extremities of archistriatum and palaeostriatum, for the cells here, although mixed in size, are on the average larger than those of the neostriatum elsewhere. Caudally and laterally from the region represented by fig. 22 this zone expands and ultimately forms the major part of the striatum.

A study of sagittal sections is very instructive at this level of the neostriatum. Fig. 28 shows the lamina medullaris dorsalis bifurcating caudally; three masses of cells are meeting here. The posterior mass of cells is the one just described and which dorsally and caudally expands to very considerable proportions and bulges into the ventricle. The superior mass contains close to the lamina itself the zone of small cells already described. Here, as previously in transverse sections, it is seen to separate the most dorsal part of the main neostriatal area from that part of it which bulges so prominently into the ventricle caudally.

Although there is undoubtedly considerable variation in the conformation and development of the various masses in different forms I think it very probable that these two zones together correspond with the neostriatum caudale of Huber and Crosby. I am not prepared to say that my two divisions of the neostriatum caudale correspond with their pars anterior and pars

posterior, because the cell grouping in *Apteryx* is different from that in the forms described by them, and also the two components of the neostriatum caudale in *Apteryx* do not bear that relationship to the lamina hyperstriatica on which Huber and Crosby based their description.

A comparison of my figures with fig. 23 of Huber and Crosby (1929) will show the rather close resemblance between this region in *Apteryx* and in the sparrow.

Ectostriatum

The ectostriatum as seen in figs. 12–22 consists of a lamina of cells lying closely associated with the lateral part of the lamina medullaris dorsalis. It thus lies between the neostriatum laterally and the palaeostriatum and archistriatum medially. Its delimitation from the palaeostriatum is as a rule readily determined owing to the main mass of the lamina medullaris lying between them; but many fibres of this lamina actually traverse the ectostriatum, so that in Weigert preparations this mass is readily picked up as a plexiform mass of myelinated fibres. The cells of the palaeostriatum and ectostriatum are not intermingled. Laterally, however, the boundary line between the ectostriatum and the neostriatum is not so clear and some admixture of the cells may be detected. But cell size and straining properties in the two masses are distinctly different, and despite what appears to be a slight overlapping they are in general fairly readily distinguishable. Throughout the greater part of its extent the ectostriatum reaches ventrally to the brain surface usually just lateral to the fissura ventralis, but near its caudal and rostral extremities it fails to do so and becomes quite an isolated island of cells buried deeply in the substance of the striatum. Dorsally it extends in a curved manner round the lateral aspect of the palaeostriatum and tapers off gradually above (figs. 16–20). Its rostro-caudal extent corresponds approximately with that of the palaeostriatum. The blood vessels which constantly enter at the fissura ventralis run dorsally in amongst the fibres of the lamina medullaris dorsalis, generally medial to the ectostriatum, but occasionally, in the caudal part of the nucleus, lateral to it.

The cells which are distinctively small and closely packed take only a light stain with toluidin blue. Medially where the fibres of the lamina medullaris are most compact the cells of the ectostriatum are relatively sparse and entirely lacking in any sort of arrangement; towards the lateral part of the nucleus the cells tend to be arranged in radiating columns. In this lateral part too there is some admixture of the larger elements of the neostriatum with the ectostriatal cells. Craigie (1930) has noted the presence of some larger elements amongst the characteristically small cells of the ectostriatum; this feature is present in my preparation too, but such large cells are very few indeed.

Although the contour of the ectostriatum in *Apteryx* is unusual (cf. Schroeder, 1912, and Huber and Crosby, 1929) there is little doubt that it corresponds with the region similarly named by these writers. Craigie has

described both a nucleus basalis and an ectostriatum in *Apteryx*, but he states that these two masses might possibly be regarded as one. His nucleus basalis is the most rostral part of the mass which I have described as ectostriatum. Of course it may be that such a division is justifiable on other criteria than the size, grouping, and staining qualities of the cells, but I certainly cannot by these means further subdivide what I have included under the heading of ectostriatum. A nucleus basalis is not therefore figured as a separate mass. It is interesting to note that certain other forms have also failed to yield evidence of a nucleus basalis (Huber and Crosby, 1929).

Hyperstriatum

Considerable difficulty is experienced in determining the limits and subdivisions of this mass in *Apteryx*. The nomenclature, moreover, has been determined by the study of other forms which show profound differences from the condition seen here. The general mass concerned corresponds clearly enough with the hyperstriatum of Huber and Crosby (1929) and with the fields *A*, *C* and *D* of Rose (1914), but subdivision of the whole mass along the lines of Rose and of Huber and Crosby is fraught with such difficulty that one prefers to make rather tentative suggestions than dogmatic statements as to the homologies. Craigie (1930) has found some evidence of subdivision along the lines of the above writers, but in several respects I must differ from him; also there are notable constant features of the hyperstriatal area in my preparations which his apparently did not show. I have of course had the advantage of studying the first available sagittal series. It must be clearly understood that the hyperstriatum of my description corresponds with the neostriatum superius of Craigie (1928 and 1930).

Figs. 16–20 show the extent of the hyperstriatum at the level where it reaches its maximum proportions. Here it forms a large C-shaped band covering the subjacent neostriatum dorsally and laterally. At its dorsomedial extremity it abuts on the ventricle where there may occasionally be detected a very slight neo-palaeostriatal fissure. Between neostriatum and hyperstriatum a narrow zone is present in which the cells are relatively sparse, the lamina hyperstriatica. Lateral to the hyperstriatum and lying close to the pia is a thin lamina of flattened cells, which lie parallel to the brain surface. This superficial zone of cells is generally considered to represent a reduced cortical remnant, and will be discussed with the cortical masses elsewhere.

Rostral to the level of the figures quoted above the contour of the hyperstriatum becomes very different in transverse sections. Figs. 27 and 28 of the sagittal series show how at its rostral extremity the whole hyperstriatum projects ventrally into the midst of the neostriatum. The levels of figs. 5, 6, 8 and 9 are indicated on fig. 26. Caudally the main change in contour of the whole hyperstriatum is brought about by a recession upwards of its lateral portion, which is accompanied by a corresponding increase in size of the neostriatum (figs. 22 and 23). Its boundaries here become less distinct.

The cells comprising the hyperstriatum as a whole are more densely staining than those of the neostriatum, and although cell size is not constant the cells are in general larger than either the neostriatal or palaeostriatal cells. Mixed with these characteristically large cells are many smaller ones. Careful inspection of the whole hyperstriatum, however, does not permit one to make such a clear subdivision into component masses on the basis of cell size as Huber and Crosby (1929) could so readily do in other forms. Three zones are traditionally ascribed to the hyperstriatum, viz. fields *A*, *C* and *D* of Rose. Huber and Crosby have named these nucleus intercalatus hyperstriati, hyperstriatum dorsale and hyperstriatum ventrale. They, moreover, found evidence of a further subdivision of the hyperstriatum ventrale, and have therefore described this mass as consisting of two zones, hyperstriatum ventrale ventroventrale and hyperstriatum ventrale ventrodorsale. Of this latter subdivision I cannot find any evidence in my preparations so that it will not be mentioned further. Such subdivisions as are evident in *Apteryx* might now be considered in detail.

There is one major component forming the greater part of what can be seen, e.g. in such figures as 13–18 (transverse) and 27 (sagittal). It might be described as the part which lies immediately upon the neostriatum and which is separated from it by the lamina hyperstriatica. This zone of cells, which will be called the hyperstriatum ventrale, is generally somewhat less in width than the neostriatum. It is composed of large densely staining cells with a slight admixture of smaller elements. The radial arrangement of the cells which was typical of the neostriatum is not evident except to a slight extent close to the lamina hyperstriatica. Nevertheless fibre preparations show a radiation of medullated fibres throughout the mass such as was also typical of the neostriatum. The distribution of the cells is fairly even. This component extends in unbroken continuity from the olfactory ventricle (fig. 4) back almost to the caudal extremity of the striatum (fig. 23). Throughout its whole extent it is related medially to the ventricle, for the rhinocele is continuous with the cavity of the main lateral ventricle. Dorsally it is related to the narrow zone of small cells, the nucleus intercalatus hyperstriati, which is evident in the majority of sections and which will be discussed below. No subdivision of this transversely placed portion of the hyperstriatum ventrale can be made, although Craigie (1930) in his fig. 6 depicts fields *C* and *D* of Rose as being evident here. He found the cells of field *C* "a little larger and more rounded" than those of neostriatum. On the contrary I find no such distinction. The most that I can possibly say is that the cells adjacent to the nucleus intercalatus hyperstriati show a tendency to be flattened parallel to the latter. But these features are so very inconspicuous, and the zone in which they occur so very narrow that I much prefer to consider this merely as the transition zone between hyperstriatum ventrale and the nucleus intercalatus hyperstriati. I therefore would not describe any hyperstriatum dorsale (Rose's field *C*) in this dorsal part of the hyperstriatum.

Laterally, however, that part of the hyperstriatum which is placed as a curved zone close to the brain surface presents modifications which allow of some agreement with Craigie's findings. Here the deeper zone of cells in the hyperstriatum is obviously continuous dorso-medially with that portion of the hyperstriatum just described. Its cell characters are the same, and there is indeed no feature to deter one from considering this deeper zone together with its continuation medially as forming the hyperstriatum ventrale of Huber and Crosby (1929) and field *D* of Rose (1914). Placed superficial to this, however, we now have a zone of cells of definite width with characteristics which render it easily capable of differentiation from the subjacent hyperstriatum ventrale. The cells are smaller than those of the latter mass and are somewhat flattened. What is perhaps most characteristic is their arrangement in rows parallel with the brain surface. In the rostral part of the hemisphere this cell mass cannot be identified, but from the level of fig. 10 caudalwards it can be clearly defined. It is generally wider at its ventral extremity, and is often reduced to a very narrow zone dorsally. Blood vessels are frequently present in it and, maybe for this reason, the cells are not infrequently disposed in numerous little flattened clumps. Overlying it and immediately subjacent to the pia is a very indefinite and narrow layer of small cells reputed to be a cortical remnant. This field of cells, corresponding clearly with the lateral part of the field which Craigie has labelled "*C*" in his figures, covers the hyperstriatum ventrale from almost its ventral extremity up as far as the point where it sweeps medialwards towards the ventricle. Here however they part company. The more superficially placed hyperstriatal zone is now generally reduced to a very thin lamina of cells and with what particular cell group it becomes continuous dorsally it is by no means easy to say. Over a considerable extent of the hemisphere there is in this region a somewhat fusiform mass of cells which lies at the lateral extremity of the nucleus intercalatus hyperstriati. Owing to their deeper stain and also to their close packing these cells stand out clearly as shown in figs. 18–22. It is with this mass of cells, if any, that the superficial component of the hyperstriatum becomes continuous. But as I have stated above the interpretation of the arrangements of the cell masses here is by no means easy; moreover, the disposition of the various masses is not typical of the avian plan as described by Rose and by Huber and Crosby. That the superficial hyperstriatal zone above described is a separate component of the hyperstriatum I have no doubt, and the appearances certainly suggest that this component becomes continuous dorsally with the aggregation mentioned. Huber and Crosby in their 1929 paper have figured the hyperstriatum dorsale at certain levels as a localised mass of cells situated very much as is the aggregation of cells which I have figured at the lateral extremity of the nucleus intercalatus hyperstriati (see figs. 6 and 9 of Huber and Crosby, 1929). This whole superficial zone of the hyperstriatum has therefore been named hyperstriatum dorsale.

Reference has already been made to the nucleus intercalatus hyperstriati,

the third component of the hyperstriatum. It lies transversely beneath the hyperstriatum accessorium. Medially it is somewhat expanded where it lies closely under the ependyma in the region of the dorso-lateral angle of the ventricle. Here it spreads ventrally for a short distance, overlapping the medial extremity of the hyperstriatum ventrale. Laterally the nucleus is considerably attenuated and ends generally in the vicinity of the aggregation of cells which has been described as forming the dorsal extremity of the hyperstriatum dorsale. Craigie (1930) has figured the mass in his fig. 6 (Rose's field *A*); but I find its development by no means so restricted as he indicates. Figs. 12–17 show it as a definite though thin lamina of very small cells. Blood vessels are frequently found in it. Rostrally in the hemisphere the mass is not found at all; it appears first at the level of fig. 9 and extends therefrom almost to the caudal end of the striatum.

Hyperstriatum accessorium

Craigie (1930) has placed his description of this mass along with that of the cortical fields, stating that "it seems only reasonable...to regard it as a reduced homologue of the pallial region which in mammals has produced the cortex cerebri" (p. 258). By placing my description of the hyperstriatum accessorium along with the other undoubted striatal components I do not thereby mean to disagree with Craigie as to the likelihood of this mass being really cortical, a conclusion, indeed, with which I find myself rather in favour. Named as it is—maybe unfortunately—the hyperstriatum accessorium will be regarded here as a component of the striatum for purposes of description.

The region involved in this description produces a rounded prominence in the dorso-medial and caudal part of the hemisphere as it is viewed from the lateral or dorsal aspect (figs. 1 and 2). This is the pars medialis or "Sagittalwulst" of the description of Ariëns Kappers (1921). Bounding it laterally and in front is the vallecula—quite a well-marked depression on the brain surface in *Apteryx*.

Within this field there are several subdivisions which are readily picked up in cell preparations, subdivisions moreover which might quite possibly be separate nuclear masses, and whose inclusion here under the one heading is made merely owing to lack of knowledge as to where else they might better be placed.

Fig. 13 shows the hyperstriatum accessorium where its extent is considerable and where nuclear specialisations are most obvious within it. The whole mass is seen to lie dorsal to the level of the angulus dorsolateralis of the ventricle—a level which can generally be recognised by the presence of the narrow nucleus intercalatus hyperstriati. At this level also conspicuous blood vessels are frequently in evidence. I follow Huber and Crosby (1929) in excluding the very thin cortical lamina of this region from the hyperstriatum accessorium. Within the zone thus demarcated three subdivisions will be described.

The first subdivision (marked "1" in figs. 11–22) lies laterally and presents a concave margin facing medially. Its cells are mostly large and take with toluidin blue a characteristically deep stain. Dorsally the cells sweep medialwards and become apparently continuous with the corticoid zone of flattened cells lying closely beneath the pia. Ventro-medially the field becomes considerably attenuated and, as a thin darkly staining zone, it extends in places to the level of the dorso-lateral angle of the ventricle. The delimitation laterally of this first component of the accessory hyperstriatum is less clear and in some places its separation from the hyperstriatum is scarcely evident. As Craigie (1930) points out, the lateral edge of the hyperstriatum accessorium tapers off into the deeper part of the thin corticoid layer which overlies the striatum laterally; but it must be understood that such corticoid layers are themselves anything but well defined.

Lying medial to the above zone and to some extent embraced by its two medial extensions is a large area containing cells less densely packed and certainly taking a lighter stain. Within this area two zones are distinguishable, one lying laterally close to the area "1" and the other placed more medially and dorsally nearer the dorso-medial angle of the hemisphere. The former of these, labelled "2," is a small-celled faintly staining mass of indefinite limits medially, though clearly separable from area "1" laterally. In area "3" small and large cells are mixed, and it is only the presence of larger cells here that allows of differentiation of "3" from "2." Taking the area under consideration as a whole one may say then that the cells are most densely packed and most deeply staining laterally, whilst medially there is a change in cell character as the cortical areas of the medial wall are approached.

These three zones within the accessory hyperstriatum are present over a considerable extent as is shown in figs. 11–12, but cannot be traced to the rostral or caudal limits of the hemisphere. Indeed the region in which they are found is confined fairly accurately to the Sagittalwulst as seen on the surface of the brain.

Rostral to the level discussed above little variation occurs for some considerable distance. The superficial corticoid zone is in places very evident, completely covering the whole hyperstriatum accessorium, and being apparently continuous with the cortical field of the medial wall. As the hyperstriatum becomes smaller definition of its constituent parts becomes less evident. Figs. 11–9 show the gradual decrease in thickness of this zone in its rostral part. I am unable to describe any continuation of the accessory hyperstriatum beyond the limits of the vallecula, so that in fig. 27 the hyperstriatum, where it approaches the brain surface so closely just rostral to the vallecula, is not covered by any continuation of the hyperstriatum accessorium, but only a very reduced cortical remnant. It has already been pointed out that in my preparations it is quite impossible to find any evidence of continuity of the hyperstriatum with the neostriatum rostrally. In transverse sections, where of course the medially curved rostral part of the vallecula

cannot be distinguished, no accessory hyperstriatum is found rostral to about the level of fig. 9. The anterior termination of this mass corresponds therefore with the restriction of the ventricle in its dorso-lateral part.

Caudally there is no alteration in the arrangement of the masses comprising the hyperstriatum accessorium, until very close to the caudal extremity of the striatum. Here the definition of the various zones is obscured, and indeed separation of hyperstriatum accessorium from neostriatum is not possible (fig. 25).

BASAL CENTRES OF THE VENTRO-MEDIAL FORE-BRAIN WALL

Huber and Crosby in 1929 gave a detailed analysis of the nuclear configuration of this region in several avian forms, more particularly the sparrow. They were able in most cases to distinguish the same essential arrangement of the centres as exists in the reptilian brain, but they say (p. 25) "on the whole these centres are not as well developed in avian as in reptilian forms, and in some birds are greatly reduced." In his account of the brain of *Apteryx*, Craigie was able to distinguish several of the ventro-medial wall constituents, but I gather that this was done with some degree of difficulty. I can find several nuclear masses allowing of easy differentiation and obviously homologous with those described for other avian forms by Huber and Crosby, but apart from these few distinct masses the remaining cells scarcely allow of classification.

In fig. 6 the area praepyriformis (area 51 of Rose) forms a narrow lamina of cells lying deep to the lateral part of the bulbar formation and extending for some distance beyond it where it lies fairly close to the pia. A similar, but less extensive, layer lies deep to the medial part of the bulbar formation and extends beyond this on the medial aspect of the striatal masses for a short distance. This medial portion is never conspicuous and further caudally blends with the more prominent lateral part. The cells of both parts are small and take a moderately deep stain with toluidin blue. As the bulbar formation diminishes towards its caudal extremity the lateral part of the area praepyriformis extends further medially to meet the medial part. Just behind the bulb (fig. 9) they form a definite cortical-like lamina overlying the ventro-medial aspect of the hemisphere. In fig. 10 two parts are apparent in the area praepyriformis; a lateral part related to the neostriatum and a medial and slightly deeper lamina underlying the palaeostriatum. The cells of the former are slightly larger than those of the more medial sheet of cells. It is quite possible that they may represent different nuclear masses, and the possibility suggests itself that the more medial one may perhaps represent a nucleus basalis, which I cannot locate as a separate entity in the position given by Craigie. The area praepyriformis is lost a short distance behind the bulb. Craigie (1930) found some slight evidence of lamination in the area praepyriformis.

Two septal nuclei are present, but these are confined ventrally and caudally in the medial brain wall. Some points of resemblance are noticeable between their arrangement in *Apteryx* and the position and general relations of the similarly named masses in the reptile *Sphenodon* (Durward, 1930). The medial septal nucleus extends furthest rostrally (figs. 12 and 13) where it is composed of a mass of rather small cells continuous below the ventral angle of the ventricle with the palaeostriatum. No differentiation can be made at such a level into separate components, although this cell group which lies in the vicinity of the ventral angle and is continuous medially with the medial septal nucleus and laterally with the palaeostriatum is doubtless the nucleus accumbens of authors. The medial brain wall above the medial septal nucleus is very thin and devoid of any cells (figs. 11–17). This nucleus continues caudally unaltered until the septo-mesencephalic tract has reached some size and displaces the septal nucleus towards the ventricular aspect of the brain wall (figs. 16 and 17). Accompanying the septo-mesencephalic tract in this region are some large cells which will be referred to later in this section.

In naming the other septal nucleus lateral I should explain that this is done not because of its position in *Apteryx* relative to the medial nucleus, but rather on the analogy of the condition in *Sphenodon*. In that reptile the septal nuclei are very distinct and bear essentially a medio-lateral relation to each other. In *Apteryx* the septal nuclei are not so distinct, and the relationship is scarcely a medio-lateral one, but the similarly named nuclei of the two forms correspond in other respects. I have therefore reversed the terms as Craigie has applied them in *Apteryx*. The lateral septal nucleus appears dorsal to the medial nucleus—as was the case in *Sphenodon*—at a level not far rostral to the foramen of Monro. It increases rapidly in size as traced caudally and soon forms a well-circumscribed mass of medium-sized cells which have a distinctly whorled arrangement. Lateral and medial nuclei are not distinctly separable from each other, and indeed as the foramen of Monro is approached they certainly are no longer differentiable. No nuclear formation is detectable caudal to the foramen (fig. 21).

In the reptile *Sphenodon* and in certain avian forms the cortical sheet of the medial wall overlapped the septal nuclei to some extent; such an arrangement is not evident in *Apteryx*. Where the two fields are closely approximated there is an appearance of a slight cell-free zone separating them.

Other cell groups are the bed nuclei of the commissures and the nucleus of the diagonal band of Broca. Of the former nothing need be said beyond that they are present and consist of very small, closely packed, and densely staining cells. The anterior commissure is strikingly large and in sagittal sections is seen to have its small-celled bed nucleus massed chiefly anterior and posterior to it. Lying dorsal and somewhat caudal to the above commissure is the very small pallial commissure, embedded in the small-celled zone lying behind the anterior commissure. In the region of the commissures there is some blending of the septal and the bed nuclei.

The nucleus of the diagonal band is shown in figs. 16 and 17 as some fairly large cells massed chiefly to the medial side of the septo-mesencephalic tract. This is quite a small nucleus and in places is not sharply delimited; some cells of a quite comparable appearance lie lateral to the tract and in front of it, blending apparently with adjacent septal cells.

CORTICAL AREAS

It is to the cortical areas of the fore-brain of *Apteryx* that most interest attaches at the present time owing largely to the very careful observations of Craigie (1929 and 1930); and it would appear that this bird certainly does present features which set it apart to some extent from many of the avian forms described by other writers. Craigie's very careful observations have been followed in my preparations, and I find myself in general agreement with the majority of his findings; points in which I differ will be mentioned under the headings of the various fields. I have also had in addition to Craigie's published accounts of his researches, a private communication from him (1930 *a*) in which he has given me his later considered views on various points regarding the cortical areas of *Apteryx*, which he now considers in a light somewhat different from that of his published works.

The hippocampal formation

Figs. 18–20 show sections in which the hippocampal formation is clearly identifiable and readily differentiated from adjacent areas. The whole formation is somewhat wedge-shaped with its apex pointing ventrally. Rose (1914) in the same region has differentiated a ventral Ammonsformation and a dorsal area "28" or entorhinal area. There can be no doubt but that these two areas together correspond to the zone treated here simply as hippocampus. Huber and Crosby (1929) agree with Rose in finding here two components, viz. hippocampus and entorhinal area; but they also indicate that the differentiation of these two masses is not well marked. In *Apteryx* there is no apparent reason in cell preparations, or so far as can yet be told in fibre preparations, for any subdivision of the area in question, and it will therefore be treated as hippocampus. In this matter I agree entirely with Craigie. The use of the terms entorhinal and para-entorhinal in the dorso-medial segment of the avian brain is unfortunate.

The cells comprising the hippocampus may be described in three zones, the lateral and medial of which are the more definite. The medial column of cells throughout its whole dorso-ventral extent is separated from the pia by a cell-free layer containing a definite tract of medullated fibres, the septo-mesencephalic tract. The cells are small and fairly closely packed. Dorsally this column presents a feature which persists at all levels and serves to identify the dorsal limit of the hippocampus, viz. the cells here are distinctly smaller than those of the column more ventrally and are packed more closely. Immediately dorsal to this specialised cell group the brain wall is acellular owing

to the lateral deviation of fibres of the septo-mesencephalic tract. At this level also Craigie (1929 and 1930) describes what he calls the fissura hippocampi indenting the medial brain wall on its pial aspect. A furrow is occasionally identifiable at this spot, particularly in sections through the caudal part of the hemisphere; further mention will be made of this feature when the more caudal part of the medial wall comes under discussion.

The lateral column of cells is also separated from the ependyma by a more or less cell-free zone, but the clear border which the whole formation presented medially is certainly not seen laterally. Cell size and density of staining do not differ appreciably from what was described for the more medially placed column, but the packing of the cells is certainly less regular and not so close.

Between these two more or less well-defined columns there is a group of cells with little definite arrangement, but tending chiefly to be associated with the medial column. Their grouping is loose and staining with toluidin blue is lighter than that of the columns between which it lies.

Ventrally there is some degree of fusion of all three columns of cells, and a rapid diminution of the whole hippocampus until the whole medial wall contains merely a lamina of fibres.

Dorsally certain features have been mentioned such as the specialised cell group at the dorsal extremity of the medial column and the cell-free area immediately above it. More deeply in the brain wall separation of the hippocampus from the field dorsal to it is not by any means clear. The cells of both areas are intermingled, and, although occasionally a suggestion of a cell-free zone may be seen between them, yet in general it is neither sufficiently constant nor marked to be classed as a feature of the region. Cell size and grouping are, however, sufficiently different in the two regions to allow of an approximate boundary line being drawn between them. In Weigert preparations the passage laterally and dorsally of a band of fibres of the septo-mesencephalic tract at this level makes the boundary evident.

Differentiation of the hippocampus from the septum is made either by the presence of a cell-free zone intervening, or where such zone is not evident, by a quite obvious difference in cell character. The cells of the septum are smaller and take a lighter stain. On the ventricular aspect also, towards the level of the foramen of Monro, a well-marked sulcus marks the junction of hippocampus and septum (figs. 18 and 19).

As traced rostrally from the level shown in fig. 18 the size of the hippocampal formation gradually decreases both in its width and in its dorso-ventral extent, but the general arrangement of its cells undergoes no essential alteration. The differentiation of hippocampus from the more dorsally situated cortical field becomes less obvious in the more rostral sections. Fig. 14 shows the rostral extremity of the hippocampus.

Considerable changes, however, are noticeable in the caudal part of the formation. Its dorso-ventral extent gradually increases by extension ven-

trally, particularly in the post-foraminal part of the medial wall. The dorsal part of the formation retains its bulky dimensions, while ventrally it becomes a very thin lamina of cells. Figs. 20–25 show the gradual increase in dorso-ventral extent and the gradual alteration in form of the whole formation. There is at first, however, no change in the arrangement of the cells within the mass into the three columns. Some deeply staining granules gradually appear in the medial column, which moreover tends to extend further ventrally than the rest of the hippocampus (figs. 18 and 19). In the post-foraminal region another cell group appears occupying the ventral part of the medial wall. This cell column, composed of small densely staining elements massed closely together, is in line with the medial column described for the hippocampus, but it clearly overlaps the latter on its medial aspect where the two columns are contiguous (figs. 23–25). This column would appear to correspond with what Huber and Crosby (1929) have labelled "primordium hippocampi of Johnston" in the sparrow, and it is the same as the mass which Craigie has labelled fascia dentata. The former writers do not, however, state their opinion as to the homology of the cell group in question, but merely note that Johnston had considered this region as forming the primordium hippocampi and that Hines regarded it as the source of the fascia dentata. I follow Craigie in naming it fascia dentata: for its relation to the hippocampus is such, and its definition from that mass so clear, that a separate name appears justifiable, and fascia dentata is preferable to primordium hippocampi. It occupies the very thin ventral part of the medial wall and at first ends free, being replaced by a fibre zone alone; further caudally the fascia dentata is continuous with a cell group of somewhat greater dimensions lying more ventrally (figs. 24 and 25). The relationship of the fascia dentata to this cell mass is stressed by Craigie, with whose observations of the cell arrangements I agree. The mass of cells in question is continuous with the fascia dentata, but the latter very distinctly descends on its medial aspect for some distance before losing its identity. It is this region which Craigie considers to correspond with the medial superposition of certain reptiles. In contradistinction to the hippocampus the cells of the fascia dentata and of the region of the superposition are markedly smaller and more densely staining. In the fascia dentata the close packing together with the dense staining of the cells gives the field the appearance of a dark band in the medial wall readily differentiable from other masses. In the region of the superposition the cells are small and fairly densely stained, but less closely packed than in the fascia dentata. There is a definite lamina zonalis overlying both fascia dentata and the superposition and continuing on further ventrally. The cell-free zone which lay deep to the fascia dentata is lost deep to the superposition (fig. 24).

Ventral again to the region of the superposition the brain wall, still thin, contains a column of densely stained small cells which are continuous with the deeper part of the superposition, but whose cell size is slightly larger. Craigie (1930) considered this to form part of the hippocampus, but it certainly

has no cell characters at all comparable with the undoubted hippocampus further dorsally. It lies closely against the ependyma at first, but swings into the middle of the brain wall as it passes ventrally. It extends in this direction as far as the thin region of the wall (figs. 24 and 25) which Craigie has considered to be the ventral continuation of his fissura hippocampi.

Other cortical fields

Over the rest of the hemisphere the condition of the cortex is very variable and in general exceedingly reduced. The structure of the cortex in two regions has become of very considerable interest owing to Craigie's observations (1929 and 1930), which led him to describe a multilaminar condition in these cortical areas. In his published works Craigie even attributed to these fields the properties of neocortex—a view which he has since modified (1930 *a*).

The first of these fields occupies the region of the dorso-medial angle of the hemisphere and corresponds clearly with the para-entorhinal area of Huber and Crosby (1929). It occupies the interval between the hippocampus ventrally and the accessory hyperstriatum dorso-laterally. Its definition from the hippocampus by means of a superficially placed cell-free area lying dorsal to the latter mass has already been stressed. At no level does it show any clear line of demarcation from the accessory hyperstriatum. Figs. 19 and 20 show typical sections of this area (marked "*p.*"), where its relations as above mentioned are readily recognisable. Rostrally it extends forwards, gradually becoming less bulky, until somewhat behind the level of the rostral extremity of the palaeostriatum it can no longer be defined from adjacent masses. Caudally it extends right to the extremity of the hemisphere, where in company with the ventricle it curves laterally and lies on the occipital aspect of the brain. Its structure does not alter appreciably except towards its rostral and caudal extremities where it gradually fades out. Before discussing in detail the cellular arrangements in this area brief mention must be made of the presence here of a transversely running fibre tract which influences the cellular configuration of the region. Mention has already been made of the septo-mesencephalic tract lying medial to the hippocampus, and how at the dorsal extremity of the hippocampus it swept laterally causing the cell-free zone which formed a prominent landmark in the medial brain wall. Some components from the tract sweep obliquely dorsally and laterally to swing round the dorsal angle of the ventricle into the accessory hyperstriatum. This lamina shows clearly in Weigert preparations, and its course is made apparent also in cell preparations due to the disposition of the cells within it.

Close to the pia and generally forming but an ill-defined layer are some small cells. Laterally these cells blend with such corticoid layer as is present over the hyperstriatum accessorium, whilst ventrally it ceases where the septomesencephalic tract curves laterally. Although in apparent continuity with the corticoid lamina overlying the accessory hyperstriatum, this layer of cells presents certain features not seen in the former. The cells in general take

rather a denser stain, and amongst them are some which are noticeably more deeply stained than the cells of the corticoid lamina elsewhere.

In the rostral part of this area the cells subjacent to the superficial layer are scattered fairly evenly throughout the brain wall, are not densely packed, and are somewhat above medium size. This group of cells, moreover, is not sharply separable from the deeper layers of the hippocampus. The laterally sweeping bundles from the septo-mesencephalic tract cause these cells to stream upwards and laterally towards the dorsal angle of the ventricle. Further caudally some changes are found. The superficial lamina of cells remains with no appreciable alteration, but the deeper cells now show some differentiation. The deepest cells, i.e. next the ventricle, are larger and considerably more numerous, and the streaming appearance is a noticeable feature. Between these two cell groups is now another layer of cells, smaller than those of the deeper layer and taking a lighter stain; intermingled with them are some fine granules and fine, elongated and very densely staining elements. This layer might be said to be characterised by the multiform nature of its cells. This condition persists caudally right to the occipital surface of the hemisphere, where gradually the arrangement is lost.

The distinction of this zone from the more laterally placed accessory hyperstriatum is not clear; the transition from the one to the other is very gradual and, as indicated previously when dealing with the accessory hyperstriatum, this zone may be regarded from the point of view of cell characters as the culmination of a change which is gradually occurring in the accessory hyperstriatum, as it is traced from lateral to medial.

What name, if any, should be applied to this particular region is a matter of some difficulty. Huber and Crosby (1929) would appear to consider it as forming probably a part of the entorhinal area of their usage and call it the para-entorhinal area; but in *Apteryx* this zone of cells is to be associated rather with the hyperstriatum accessorium than with the hippocampus. Craigie (1930 *a*) now considers it to be para-hippocampal cortex, and certainly such a name is at least topographically suitable. With the paucity of fibre preparations available and also in fear of adding perhaps unnecessarily to an already overcrowded terminology I prefer to regard this zone as the medial extremity of the accessory hyperstriatum and certainly as more closely associated with the accessory hyperstriatum than with the hippocampus.

Craigie (1929 and 1930) has stressed the presence of a hippocampal fissure corresponding to the level of the dorsal extremity of the hippocampus of this description. I find occasionally in the caudal part of the medial aspect of the hemisphere a very slight indentation of the brain wall at this spot (fig. 20). Nowhere is it well marked, and it certainly cannot be located at all levels throughout the hippocampus; nevertheless the presence of this fissure in association with the hippocampal formation is of great interest. Huber and Crosby (1929) have found that the dorsal limit of the entorhinal area in certain forms is delimited at times by a slight sulcus. This undoubtedly corresponds

with the fissure which Craigie has noted in *Apteryx* at the dorsal limit of the hippocampal formation.

Of greater interest still is the statement by Craigie that the hippocampal fissure curves round in its caudal part and comes to lie on the medial brain wall caudo-ventrally. The depression in this latter situation is a much more definite feature than was the dorsal fissure. It is shown in figs. 24 and 25, where it coincides with the very thin part of the brain wall ventro-medially. This depression, moreover, is a constant feature in all series. But as to the continuity of these two fissures I am not at all certain; neither in transverse nor in sagittal series have I been able to satisfy myself that they really form one curved fissure. If they are not continuous then they fail to possess some of the significance attached to them by Craigie. There is no doubt, however, that the brain wall immediately lateral to this ventral depression presents rather striking evidence of lamination. The laminated area is shown in the most ventral part of the hemisphere in figs. 24 and 25, and a microphotograph of a portion of this region is reproduced in fig. 32 for comparison with Craigie's fig. 2 in his 1929 paper. After the most minute comparison of my material with Craigie's text and figures I must agree completely with his finding concerning a multilaminar condition here. The area in which it is found skirts along the ventral and caudal extremity of the lateral ventricle and lies typically just beyond the limits of the ventricle. It does not extend far laterally before its definite laminated arrangement gradually gives place to the corticoid lamina which overlies the striatum.

The lamina zonalis is relatively narrow and contains a few irregularly scattered cells. Subjacent to this is a fairly thick layer of small cells giving the appearance of a densely staining band. Generally one cannot subdivide this layer further, but in certain places there is a rather vague appearance of a division of the main mass into two layers. The deeper of these two layers presents small clumps of cells rather than a continuous sheet. Craigie has included within this sheet of cells his laminae II and III. The next zone contains medium-sized cells taking a very faint stain, in striking contrast to the last described layer which stained deeply. These two layers although containing cells of obviously different appearances are not sharply separable owing to some intermingling of the cells. The last mentioned zone, Craigie's lamina IV, is thick medially and tapers off laterally until quite soon it is indistinguishable. Following on this again is a zone containing cells rather larger and more densely staining. These cells are discrete and of uniform size. This I take to correspond with Craigie's lamina V. His lamina VI would appear to be not sharply separable from lamina V. The only evidence I can find for the description of another layer is the presence in the deepest part of this zone of some very small and densely staining cells mingled with the more typical larger ones.

Deep to this laminated zone lies the neostriatum and more medially the angle of the ventricle (figs. 24 and 25). A blood vessel is frequently detected

coursing parallel with the surface and immediately subjacent to the deepest layer.

Apart then from minor differences Craigie's and my accounts of this area are virtually identical. I cannot describe so minutely the exact nature of the cells of the various laminae, as he has done, but in the general arrangement of the laminae we are undoubtedly in agreement.

The above account represents the condition of quite a small portion of the surface of the hemisphere; when traced caudally or rostrally from the level shown in figs. 24 and 25 the various laminae soon lose their definition and fade into a non-laminated layer. Laterally, however, some semblance of lamination persists for some distance round the lateral aspect of the hemisphere, and indeed this lateral extension of the laminated field may be traced further rostrally than can the main ventral field itself. Nowhere, however, is the lamination nearly so distinct as in the ventral field.

Over the remaining parts of the cerebral hemisphere the cortex is extremely reduced and whether the term cortex is applicable depends, as Huber and Crosby say, on how it is defined. If lamination and the presence of pyramidal cells are to be considered as essentials then there is little if any true cortex anywhere in the hemisphere, and most fields would be better named corticoid.

What has been called the dorso-lateral surface area by Huber and Crosby (1929) is present in *Apteryx* over a very considerable part of the dorsal and lateral aspects of the hemisphere. Its thickness is never great, and the cells comprising it are few and flattened and lie closely under the pia. Reference has been made to this superficial zone in connection with various other cell masses, where it was seen at times to become continuous with fields which might more aptly be classed as cortical. Its separation from the underlying hyperstriatum and hyperstriatum accessorium is never well defined, and indeed in places, particularly the dorso-lateral region of the hemisphere, fusion of all the three fields mentioned is so complete that definition of any boundary line is scarcely possible.

There is one field of cells of remarkable constancy which is shown in figs. 10–21 lying superficially on the ventro-lateral aspect of the hemisphere. It is clearly delimited by a cell-free zone from the subjacent hyperstriatum. As shown in fig. 20 it reaches considerable proportions, and thereafter becomes less and less readily identified. Dorsally it tapers off, but ventrally at times it would appear to become continuous with the peri-amygdalar cortex. Its cells, however, are smaller and much less densely stained than those of the latter mass. No similarly placed zone is described for other forms, and Craigie's figures do not indicate such a field in *Apteryx* distinct from subjacent ones. If it be part of the dorso-lateral surface area then it most certainly is unique in its size and clear delimination from subjacent masses. Another possibility is that it may be a component of the hyperstriatum. The most superficial cells in this zone are more sparse and somewhat smaller than the deeper ones, but no evidence of lamination within it can be found.

Craigie (1930) has described a pyriform cortex, or, as he now prefers to name it (1930 *a*), a peri-amygdalar cortex, overlying the archistriatum. There is a very definite lamina of cells in the region which Craigie has shown in fig. 16 of his 1930 paper. This is a fairly well localised corticoid field overlying the ventral part of the archistriatum and the adjacent part of the neostriatum from both of which it is clearly separated by a cell-free zone. A microphotograph of this region is reproduced in fig. 33. Laterally it comes into contact with the specialised portion of the dorso-lateral surface area described above, but there is no admixture of the cells of the two groups. Further, the nature of the cells in the peri-amygdalar zone is quite different from those of any other group with which it lies in contiguity. I cannot detect the lamination which Craigie describes. Medially it comes into contact with the parvocellular archistriatal component, but again, though no cell-free zone separates them, cell size and staining are adequate for their differentiation.

A careful search has been made for evidence of the rhinal fissure which Craigie located in his material bounding this field laterally, but no such fissure is to be found in my material. Blood vessels not infrequently enter the brain through the peri-amygdalar zone, but apart from any localised distortion thus caused the surface presents a perfectly smooth contour in all series.

DISCUSSION

The remarkable disproportion between the obvious cortical fields and the striatum must remain perhaps the most outstanding feature of the avian hemisphere. In *Apteryx* the great reduction of the ventricle leads to the appearance of an even greater striatum than some other forms possess; but almost certainly the line of the lost ventricular extension corresponds with the position of the nucleus intercalatus hyperstriati, so that the accessory hyperstriatum would therefore be the equivalent of a cortical field contiguous medially with the hippocampus. Kuhlenbeck (1924) certainly regards the accessory hyperstriatum as homologous with part of the dorsal cortex in other forms, and Rose's field *A* he would appear to consider as representing the lateral cortex, i.e. the nucleus intercalatus hyperstriati of this description. Elliot Smith (1919 and 1919 *a*) has shown how the striatum is largely of cortical origin, and he suggests that much of what commonly goes by the name of striatum in the bird is cortical both in origin and in fibre connections. Certainly this would account for the very great disparity in the cortical fields of such forms as the kiwi and the reptile *Sphenodon*. Despite its torpidity and sluggishness *Sphenodon* has remarkably extensive cortical fields, whereas the kiwi, with active habits and apparently higher cortical powers, is remarkable for the absence of fields which can, in the usual way, be classed as cortical. The detailed knowledge of the fibre connections of the various striatal components in *Apteryx* must await further studies with enhanced material.

Huber and Crosby have found evidence in support of the interpretation of the hyperstriatal areas as vicarious cortex.

Craigie has made most interesting speculations concerning the archipallial fields of *Apteryx*. Although I failed to satisfy myself of the presence of certain fissures in other parts of the brain, e.g. amygdaloid fissure and rhinal fissure, I agree that Craigie's observations on his "hippocampal" fissure are supported at least in part in my preparations. Huber and Crosby also describe in this region a fissure which appears to correspond with Craigie's, and there can be no doubt that several avian forms at least do show a fissure of some constancy in the dorso-medial region of the hemisphere and related to the hippocampal formation. As to the naming of this fissure much must depend on the interpretation of the cortical masses underlying it. In *Apteryx* it lies at the dorsal limit of the hippocampus and corresponds with the point where the main mass of the septo-mesencephalic tract turns rather abruptly to run down the medial brain wall. Dorsal to the fissure lies the medial extremity of the accessory hyperstriatum which Craigie now considers to be true para-hippocampal cortex. The fissure mentioned by Huber and Crosby lies between the entorhinal and para-entorhinal areas of their usage; but since they consider these masses to be in reality hippocampal association cortex there is no essential difference between Craigie's and their interpretation of the cell masses in this region. Huber and Crosby, however, have not applied the name hippocampal to this furrow.

It is doubtful if such a name is justified in the bird, because its use implies its homology with the hippocampal fissure of mammals, and I am not satisfied that its position in the avian brain is such as to permit of this assumption. Its wide removal from the fascia dentata would alone render doubtful any homology with the hippocampal fissure of mammals. For these reasons I have named it the pseudo-hippocampal fissure.

The curved outline of this pseudo-hippocampal fissure which Craigie stresses with the consequent similar arrangement of the hippocampal formation would appear all the more remarkable in view of the extremely feeble development of the so-called pallial commissure; but again I cannot find satisfactory evidence of such a curved arrangement of the fissure and formation as Craigie has described. Further studies with more material sectioned in all planes, together with dissections and reconstructions, would be necessary to verify or disprove his contention.

SUMMARY

1. There are two separate and well-developed olfactory bulbs lying sessile on the rostral part of the basal aspect of the hemispheres. There are no structural peculiarities in the olfactory bulbs. The olfactory ventricle is continuous by a narrow communication with the lateral ventricle.

2. Lateral and medial septal nuclei are present in a form less well marked

than, but certainly comparable with, the septal nuclei in the para-terminal body of the reptile *Sphenodon*. Other centres of the ventro-medial brain wall are poorly developed.

3. The palaeostriatum presents a subdivision into a very conspicuous palaeostriatum augmentatum and an extremely poorly represented palaeostriatum primitivum.

4. The archistriatum is large and contains several components of which the main one, the pars magnocellularis, may itself be further subdivided on the basis of cell size. The two other components are named the pars parvocellularis and the pars ventralis; the former is probably the nucleus taeniae of authors, while the homology of the latter is not known.

5. The ectostriatum is well developed and of somewhat different form from that in many other birds where it forms a localised mass of cells buried deeply in the hemisphere. In *Apteryx* it forms rather a crescentic band covering the palaeostriatum and in part the archistriatum on their lateral aspects. Its cells are predominantly small.

6. Although as a whole well developed the neostriatum does not present all the classical subdivisions of Rose. A caudal specialisation, however, is thought to correspond with the neostriatum caudale of Huber and Crosby and probably with the field G_2 of Rose.

7. The hyperstriatum is not entirely typical of the condition described for other avian forms; but the main components of the Huber and Crosby description can be identified, viz. hyperstriatum dorsale, hyperstriatum ventrale, and nucleus intercalatus hyperstriati. A specialised cell group within the hyperstriatum dorsale has been noted.

8. The hyperstriatum accessorium is a conspicuous mass and is confined to the region of the Sagittalwulst which is bounded on the surface of the brain by a distinct vallecula. Within this mass are certain subdivisions not previously described.

9. The cortical fields, to which such great interest attaches owing to the observations of Craigie, have been described in some detail. General agreement has been reached with his findings, including his description of an area in which laminated cortex is present. The various fissures which Craigie located have not all been evident in the material at my disposal, but a fissure is certainly present at the dorsal limit of the hippocampus and corresponds with the hippocampal fissure of Craigie's usage. The term hippocampal, however, is not considered appropriate in this particular situation in the bird, and it is therefore named pseudo-hippocampal—a term which is suitable topographically and does not imply homology with the hippocampal fissure of higher forms.

A very distinct corticoid layer overlies the ventral aspect of the archistriatum and the adjacent part of the neostriatum. This is the pyriform cortex of Craigie's published works, or the peri-amygdalar cortex of his later communication (1930 *a*).

The corticoid covering (dorso-lateral surface area of Huber and Crosby) to the hemisphere in general is very poorly developed, but ventro-laterally a specialisation of this area has been described which does not appear to have been figured for other avian forms or previously for *Apteryx*.

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ABBREVIATIONS USED IN FIGURES

ang.dors.vent.	Dorsal angle of ventricle.
ang.d.l.vent.	Dorso-lateral angle of ventricle.
area praepyr.	Area praepyriformis.
archistr.mag.	Archistriatum, pars magnocellularis.
archistr.parv.	Archistriatum, pars parvocellularis.
archistr.vent.	Archistriatum, pars ventralis.
ch.	Optic chiasma.
com.ant.	Anterior commissure.
ektostr.	Ectostriatum.
ex.gran.	External granular layer.
f.	Foramen of Monro.
f.d.	Fascia dentata.
fiss.neo-palaeostr.	Fissura neo-palaeostriatica.
fiss.vent.	Fissura ventralis.
glom.	Layer of glomeruli.
hip.	Hippocampus.
hyperstr.	Hyperstriatum.

hyperstr.acc.	Hyperstriatum accessorium.
hyperstr.dors.	Hyperstriatum dorsale.
hyperstr.vent.	Hyperstriatum ventrale.
in.gran.	Internal granular layer.
lam.hyperstr.	Lamina hyperstriatica.
lam.med.dors.	Lamina medullaris dorsalis.
lat.sep.n.	Lateral septal nucleus.
med.sep.n.	Medial septal nucleus.
mit.	Layer of mitral cells.
neostr.	Neostriatum.
neostr.caud.	Neostriatum caudale.
n.accumb.	Nucleus accumbens.
n.d.b.	Nucleus of diagonal band of Broca.
n.int.hyperstr.	Nucleus intercalatus hyperstriati.
n.sep.med.	Nucleus septi medialis
olf.b.	Olfactory bulb.
olf.vent.	Olfactory ventricle.
p.	"Para-entorhinal area" of Huber and Crosby.
palaeostr.	Palaeostriatum.
palaeostr.aug.	Palaeostriatum augmentatum.
palaeostr.prim.	Palaeostriatum primitivum.
pseudohip.fiss.	Pseudo-hippocampal fissure.
tr.olf-hab.	Olfacto-habenular tract.
tr.sep-mes.	Septo-mesencephalic tract.

EXPLANATION OF FIGURES

Figs. 1, 2 and 3. Three photographs of the brain of the kiwi (G.B. 95). The scale is marked in centimetres.

Figs. 4–25. Transverse sections of the hemisphere from rostral to caudal (G.B. 81). All figures have been drawn from projections of the actual sections. Cell size is not strictly to scale, but variations in size have been indicated. The method of representing the fibre laminae is conventional. Where other portions of the brain have been lying in close proximity to the hemisphere they have been indicated merely in outline. All drawings are to the same scale.

Figs. 26–31. Outlines of sagittal sections of the hemisphere (G.B. 95) made from projections of the actual sections. The order of the figures represents their order from medial to lateral in the brain. All drawings are to the same scale. In fig. 26 the approximate levels of figs 5, 6, 8 and 9 are indicated.

EXPLANATION OF PLATE I

Fig. 32. Microphotograph of laminated cortex.

Fig. 33. Microphotograph showing peri-amygdalar cortex.

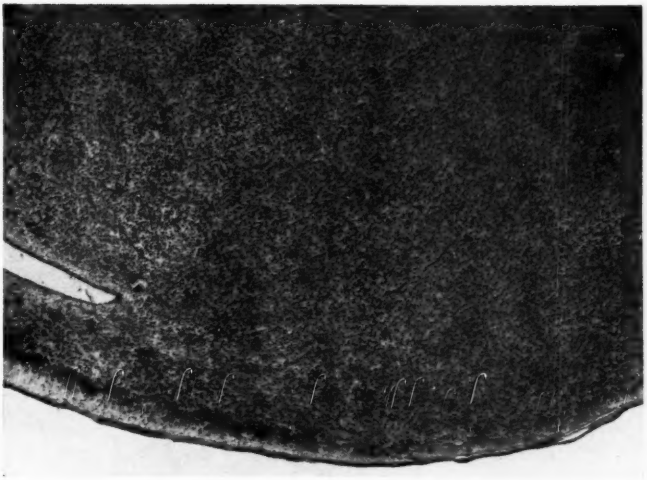

Fig. 32.

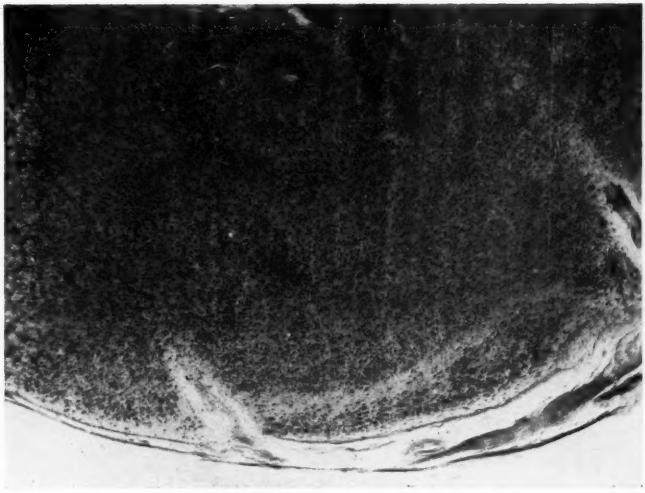

Fig. 33.

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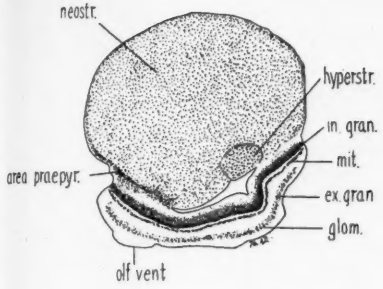

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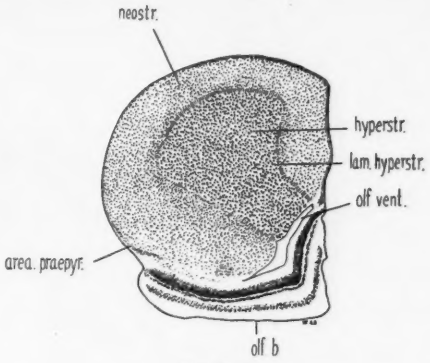

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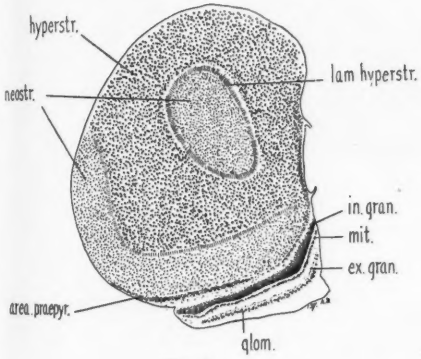


Fig. 6.

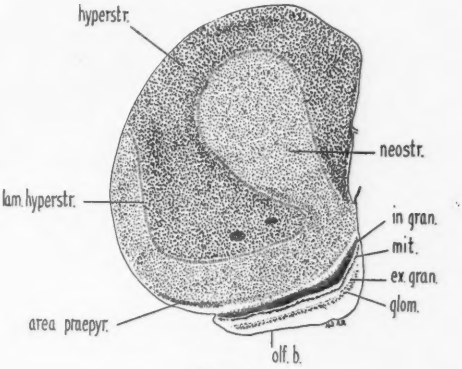


Fig. 7.

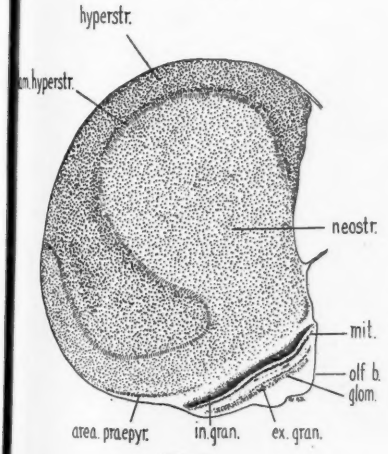


Fig. 8.

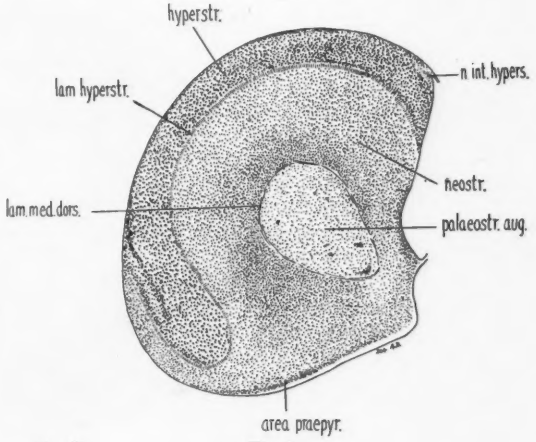
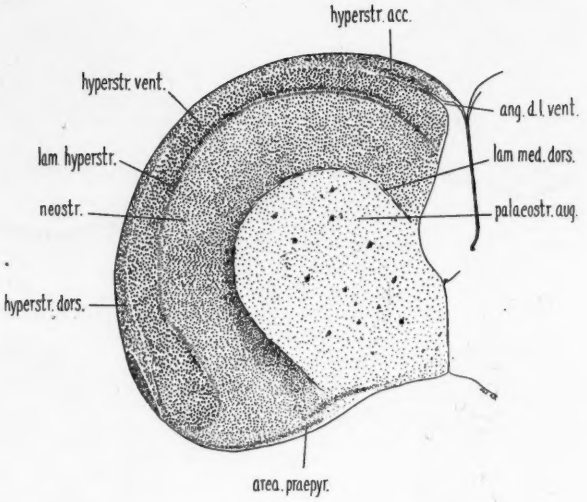


Fig. 9.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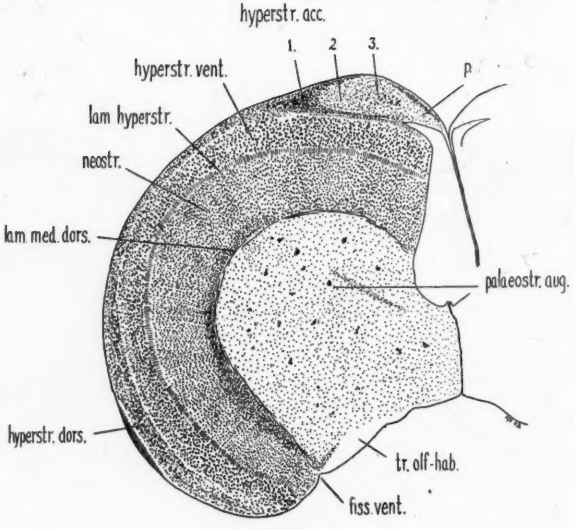
hyperstr. vent.
ang. d. l. vent.
lam hyperstr.
lam med. dors.
neostr.
palaeostr. aug.
hyperstr. dors.
area. praepyr.

Fig. 10.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1.
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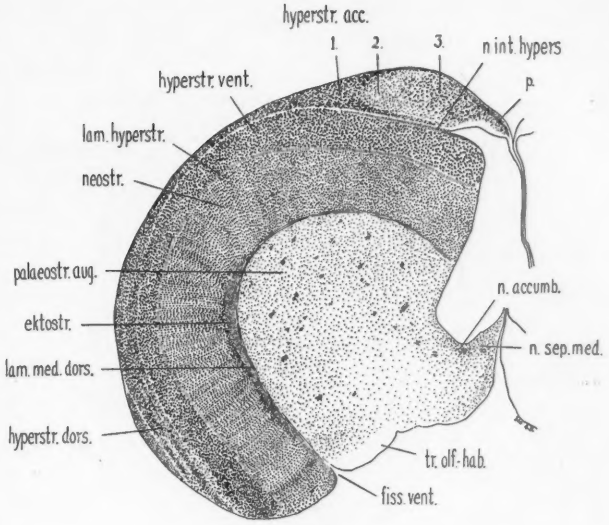
tr. olf-hab.
fiss. vent.

Fig. 11.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lam. med. dors.
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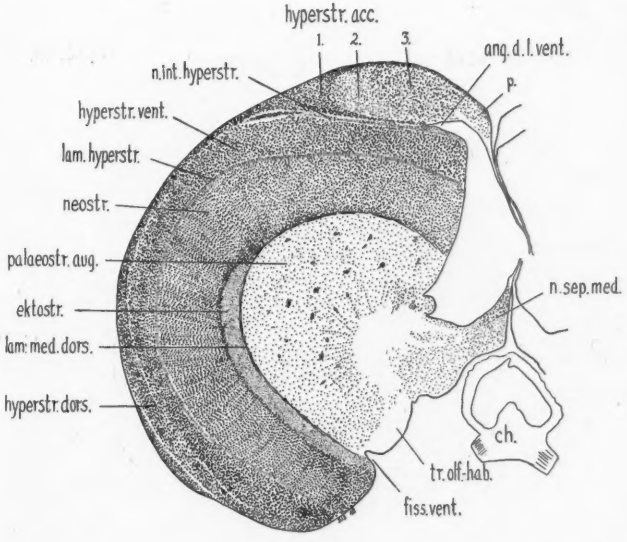
n. accumb.
n. sep. med.
tr. olf-hab.
fiss. vent.

Fig. 12.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palaeostr. aug.
ektostr.
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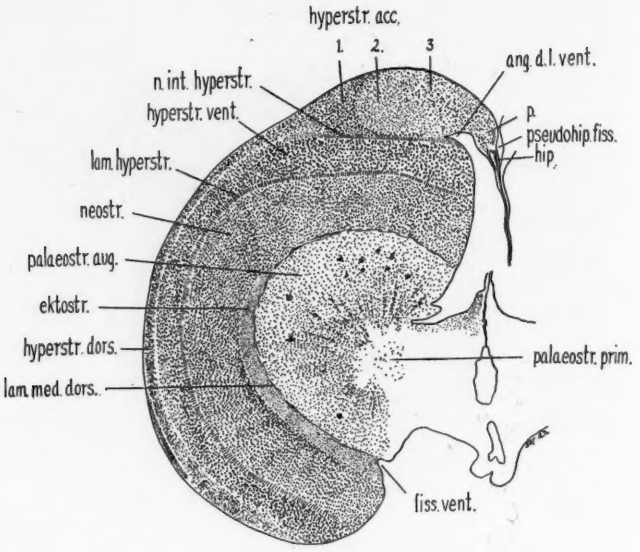
n. sep. med.
ch.
tr. olf-hab.
fiss. vent.

Fig. 13.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3
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hip.
lam. hyperstr.
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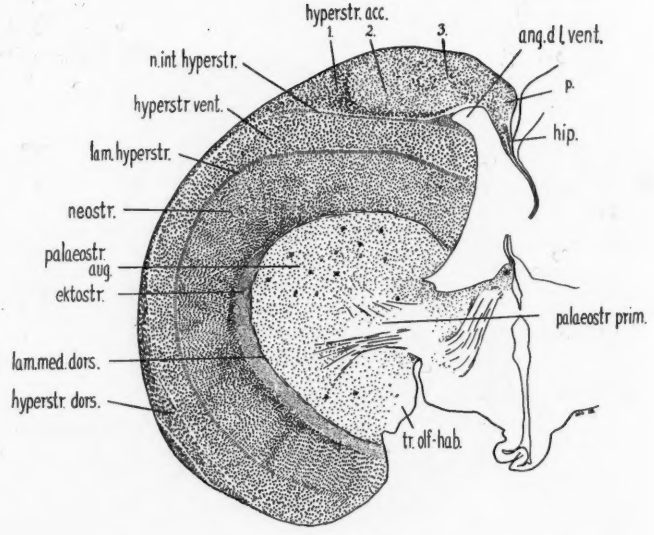
palaeostr. prim.
lam. med. dors.
fiss. vent.

Fig. 14.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2.
3.
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p.
hyperstr vent.
hip.
lam. hyperstr.
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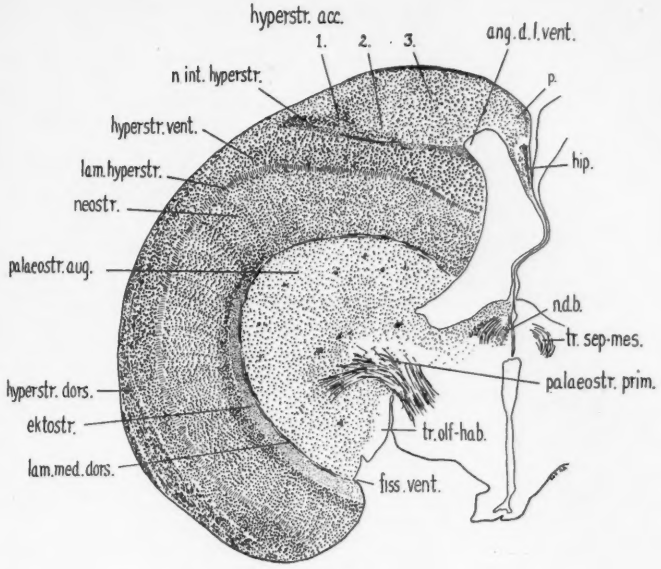
palaeostr.
aug.
ektostr.
palaeostr prim.
lam. med. dors.
hyperstr. dors.
tr. olf-hab.

Fig. 15.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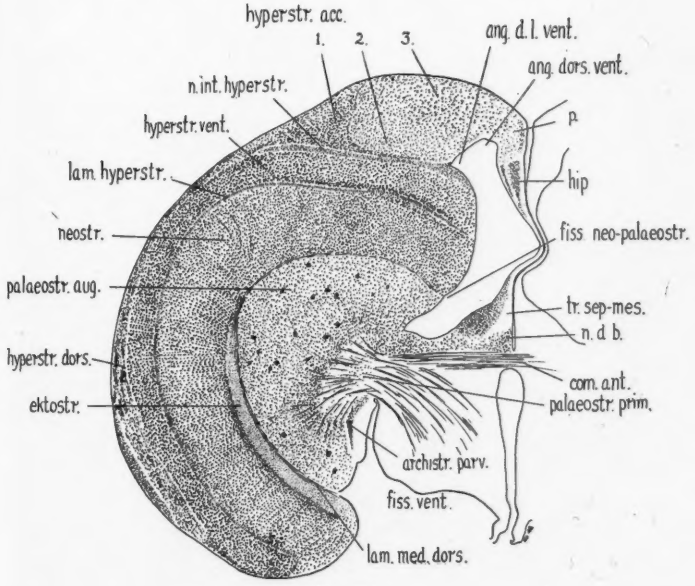
lam. med. dors.
fiss. vent.

Fig. 16.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neostr.
palaeostr. aug.
tr. sep-mes.
n. d. b.
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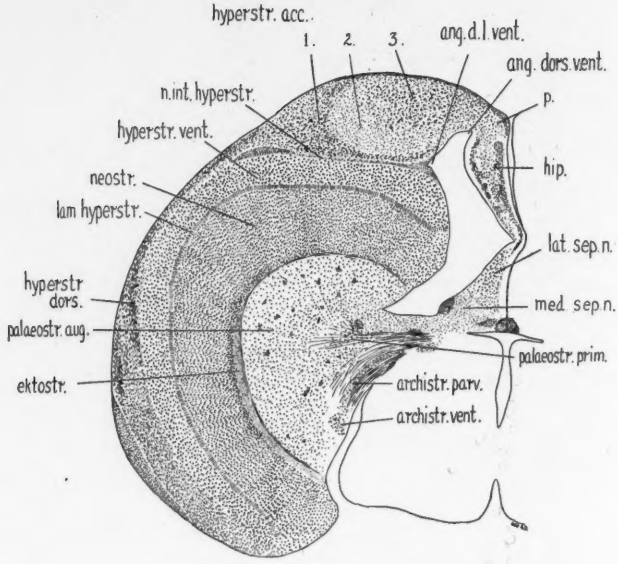
lam. med. dors.

Fig. 17.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lat. sep.n.
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archistr. vent.

Fig. 18.

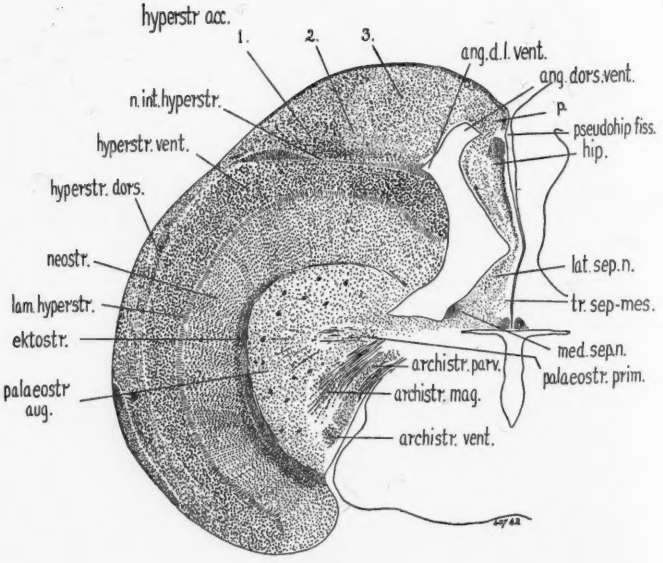
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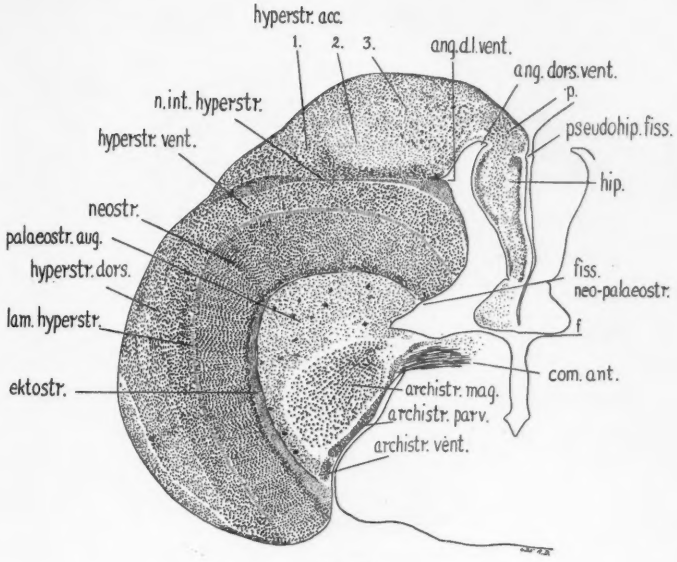
Fig. 19.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lam. hyperstr.
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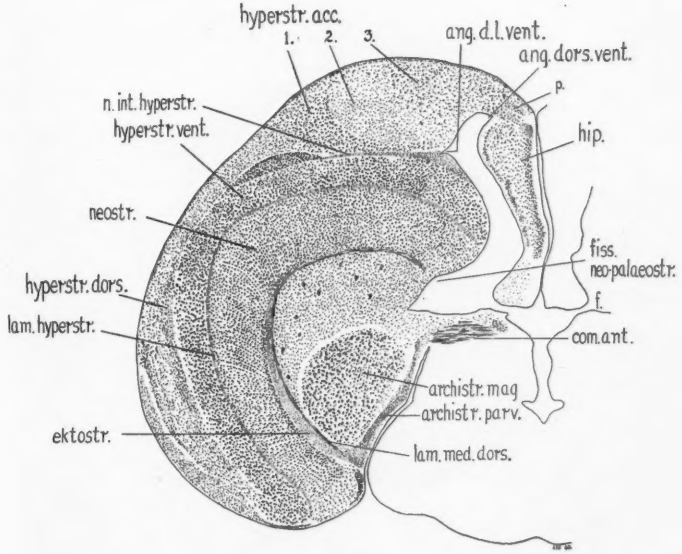
com. ant.
archistr. mag.
archistr. parv.
archistr. vent.

Fig. 20.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hyperstr. dors.
lam. hyperstr.
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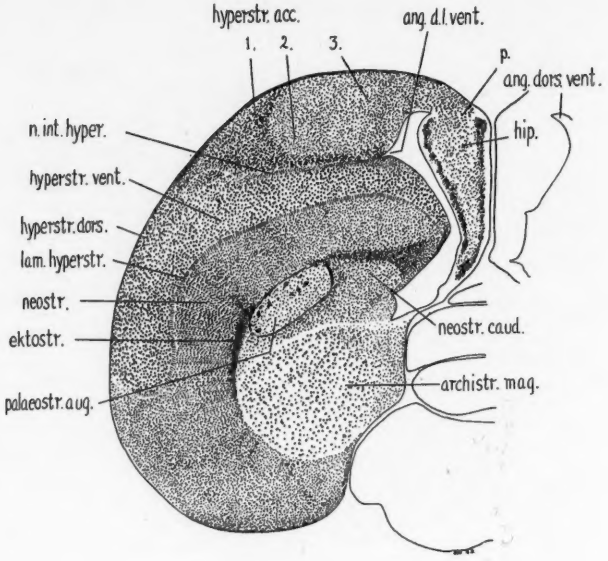
com. ant.
archistr. mag
archistr. parv.
lam. med. dors.

Fig. 21.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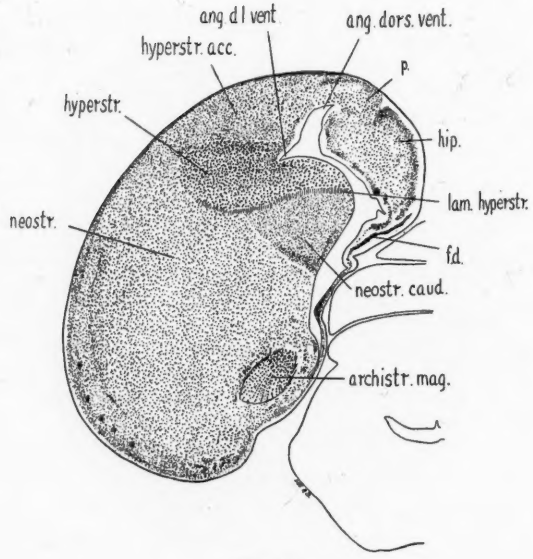
lam. hyperstr.
neostr.
ektostr.
palaeostr. aug.
neostr. caud.
archistr. mag.

Fig. 22.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f.d.
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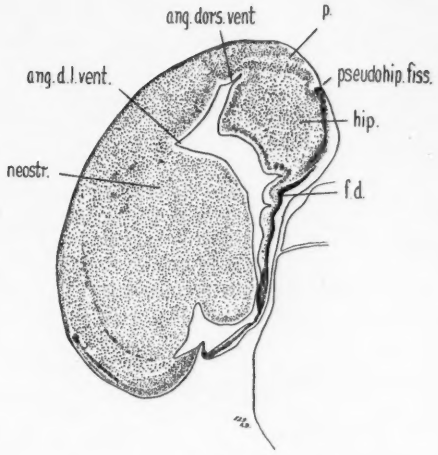
archistr. mag.

Fig. 23.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pseudohip. fiss.
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f. d.

Fig. 24.

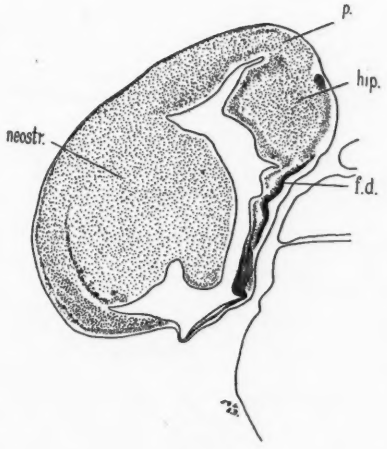

p.
hip.
neostr.
f. d.

Fig. 25

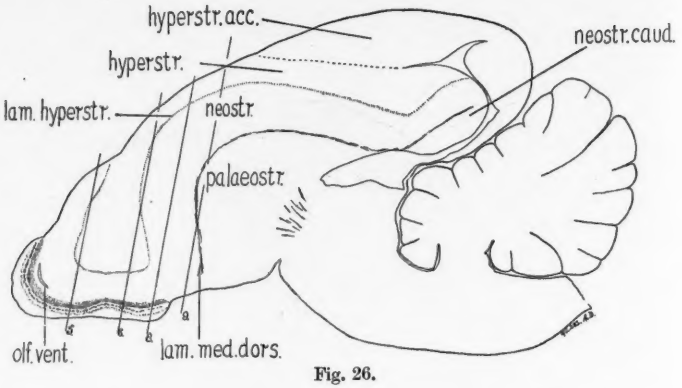


Fig. 26.

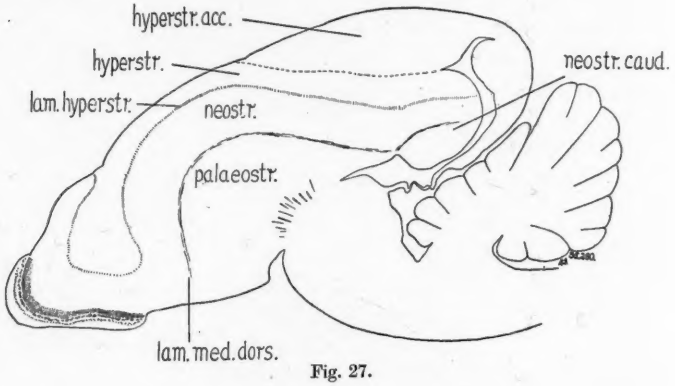


Fig. 27.

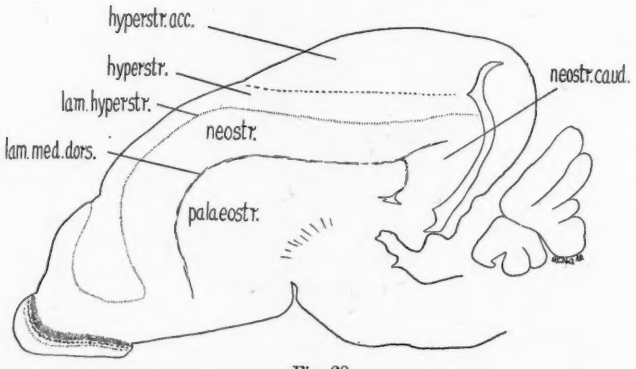
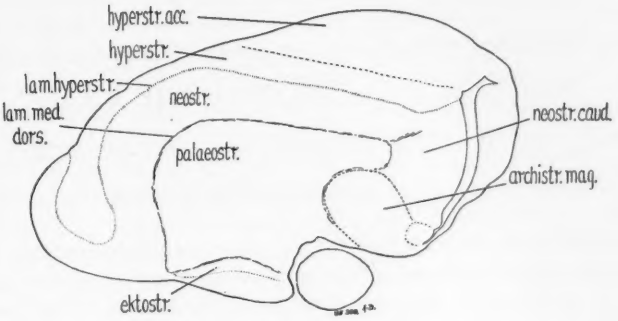


Fig. 28.



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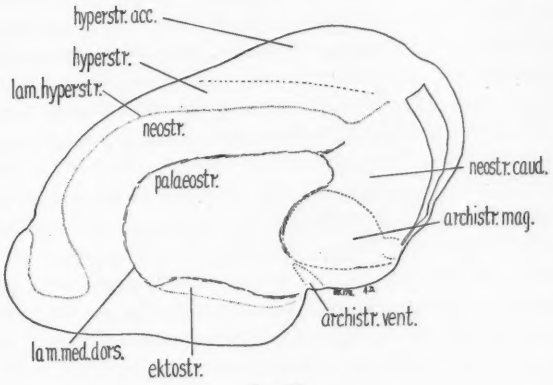

Fig. 30.

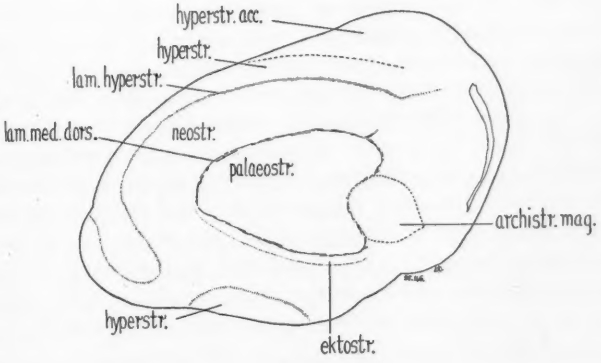

Fig. 31.

ON THE NATURE OF CERTAIN MACROSCOPIC STAINING REACTIONS OF THE BRAIN*

By D. M. BLAIR, F. DAVIES and E. W. McCLELLAND

From the Departments of Anatomy and Chemistry, King's College, London

The possibility of differentiating the grey from the white matter of the brain by the prussian-blue reaction, in thick sections for macroscopic study, has been known for many years. Interest in this use of the prussian-blue reaction has been revived in recent years by Sincke (1), who in 1926 described a technique in detail and discussed tentatively the reason for the sharp colour differentiation between the grey and the white matter. The process consists essentially in immersing thick sections of formalin-fixed brain first in a solution of a ferric salt and then, after washing, in a solution of potassium ferrocyanide; the grey matter stains intensely blue while the white matter, in a successful preparation, remains white or takes on only a faint blue tinge. Our present paper deals with the reason for the differentiation in this and other colour reactions of the brain.

Sincke is definitely of the opinion that the coloration of the grey matter in the prussian-blue reaction is due to a specific affinity between the grey matter and the iron of the solutions employed. That iron and no other metal possesses this affinity he regarded as probably proved for the reason that he failed to secure colour differentiation by the use of solutions of a number of metallic salts which react to give coloured precipitates. He specially mentions five pairs of reactants with all of which he obtained only a diffuse colouring of the whole section by the respective coloured precipitates. With nickel salts, however, the grey matter stained slightly more deeply than the white matter; this Sincke explains as probably due to the close chemical relationship between nickel and iron. He was unable to establish whether or not this supposed affinity between the grey matter and iron had any relation to iron already present in the brain substance, but admitted that physico-chemical differences between grey and white matter might contribute to the colour contrast. In particular he suggested that the presence of lipoids in the white matter would favour the adherence there of the solutions used much less than in the grey matter, because, as he demonstrated, removal of the lipoids by treatment with ether and alcohol results in a diffuse colouring of the sections when they are subsequently treated with the metallic salts. He suggested also that the alkaline nature of neuroglia might contribute to the colouring in that the interaction of neuroglia and the ferric chloride would probably result in the formation of an hydroxide of iron which subsequently reacted with the

* The authors wish to acknowledge gratefully a grant from the Publication Fund of the University of London towards the cost of the illustrations in this paper.

potassium ferrocyanide to form prussian blue. Finally, he thought that the pronounced abundance of capillaries in the grey matter might play a part.

Mulligan (2) recently described a technique for colour differentiation of the grey and the white matter of the brain through the interaction of tannic acid and an iron salt. The reason for the selective staining of the grey matter is not discussed, but it is interesting to note that enhanced colour contrast was obtained by forming on the surface of the white matter a protective film of lipoids dissolved out from the myelin sheaths of its nerve fibres.

In considering this problem of the differential staining of the grey matter by prussian blue, the absence of any positive reason which we could discover to explain a special affinity between the grey matter and iron salts led us to attempt differential staining involving the use of other than iron salts, as Sincke had done. To our surprise, in view of his failure, we succeeded with several different reactants in producing colour contrasts as marked as with the prussian-blue method. Sincke's failure may have been due to over-long immersion of the brain sections in the reagents, if the times he used at all resembled those given in his prussian-blue technique. There, he recommended soaking in ferric chloride solution for several hours; we obtained as good or better contrast after immersion for 30 seconds. In the other colour reactions which we tested, times of at most only a few minutes were found requisite for success.

In justice to Sincke it must be emphasised that in investigating these reactions we were concerned only to produce a good colour contrast and not at all to secure permanence of appearance. The short times we used, indeed, do not make for permanence. In this connection, in view of complaints of fading which have been made against specimens stained by Sincke's prussian-blue method, it may be worth noting that sections prepared by one of us strictly according to Sincke's technique have preserved their colour undimmed over a period of 4 years.

The reactions which gave us successful results are briefly described hereunder. Details of solutions and times employed are given in the appendix to this paper. In each case thick sections of formalin-hardened brain were immersed in a solution of the first-named reactant and then, after washing, in a solution of the second-named reactant. The optimum result in each reaction left the white matter unstained, or only slightly tinted with the colour which appeared strongly in the grey matter.

Reaction 1. Sodium sulphide and cobalt nitrate. The precipitation of cobalt sulphide colours the grey matter greyish-black (Plate I, fig. 1).

Reaction 2. Sodium sulphide and lead nitrate; precipitation of lead sulphide; grey matter coloured dark brown (Plate I, fig. 2).

Reaction 3. Tartar emetic and hydrogen sulphide; precipitation of antimony sulphide; grey matter coloured orange yellow (Plate I, fig. 3).

Reaction 4. Lead nitrate and potassium iodide; precipitation of lead iodide grey matter coloured canary yellow (Plate II, fig. 3).

The successful differentiation of the grey matter by these reactions made it clear that any affinity possessed by iron was shared by several other metals and strongly suggested, indeed, that no special chemical reaction at all took place between the grey matter and the various metallic salts used. To test the possibility of differentiation by a non-metallic colour reaction, the well-known starch-iodine reaction was then tried. The result was successful.

Reaction 5. Soluble starch and solution of iodine in potassium iodide; grey matter took on the characteristic dark purple colour of the starch-iodine reaction, while the white matter became a pale yellow colour (Plate II, fig. 1).

The diversity of the reactants which have thus been successfully employed to differentiate the grey and the white matter appears to exclude the likelihood of chemical combination of the reagents used with material present in the grey matter only, or present there in excess. There seems to be no chemical reason why, for example, the presence of excess of iron in the grey matter over that in the white matter should influence chemically the differentiation of the two tissues by the prussian-blue reaction. Since the specimen to be pigmented is soaked in ferric chloride, the amount of iron in the tissues due to this treatment must be much greater than any initially present and it is highly improbable that the reaction of any iron initially present should have an appreciable effect on the colour of the end result. Similarly with the other reactions employed. The summation of the evidence of all these different reactions, each improbable on the basis of a chemical combination between the grey matter and the reactants, effectively disposes of such an explanation.

The simplest and most adequate explanation of the results obtained seemed to us to be different degrees of penetration and adsorption of the reagents in the grey and in the white matter. This is supported by the fact that prolonged washing removes the first reagent used in each pair and no colour reaction follows the addition of the second reagent; and that, on the other hand, insufficient washing prevents good differentiation, the white matter being stained almost as deeply as the grey. Further, in specimens where the white matter is tinted faintly with the colour assumed by the grey matter, different depths of colour penetration in the white and grey matter are visible to the naked eye on cross-sectioning the brain slice. Concentration of solutions appears also to be a factor in the degree of differentiation.

This physical explanation of the colour differentiation was put to test by abrading localised areas of the white matter on the surface of the brain slices, by rubbing with sandpaper or stippling with a bristle brush, before applying the prussian-blue reaction. The abraded areas of white matter then pigmented almost or quite as heavily as the grey matter (Plate II, fig. 2). While drawing up this paper, subsequent to the presentation of our findings at a meeting of the Anatomical Society in June, 1931, we discovered that Mainland (3) had already (in 1928) demonstrated the possibility of producing the prussian-blue colour reaction of grey matter in abraded areas of white matter, in the course of experiments he made with Sincke's prussian-blue method. On this evidence

Mainland concluded that the colour differentiation in Sincke's method depended on the different textures of the grey and the white matter of the brain.

It is highly probable that both penetration and adsorption play a part in the colour differentiation. When a slice of brain is immersed in a solution of the first reactant, penetration of the solution takes place and also adsorption of the salt. Owing to the marked difference in the physical and chemical constitution of the grey and the white matter, it is to be expected that the rate of penetration in the two tissues and the degree of adsorption at their superficial and deep surfaces will likewise be very different. Further, the subsequent washing will remove the penetrated and adsorbed material from the two tissues at different rates. Excessive washing will remove it entirely from both tissues, but less washing removes it from the white matter only or leaves a markedly different amount in each. When the incompletely washed section is then immersed in the second reactant, precipitation of the coloured compound takes place. Adsorption probably plays a part in the retention of this precipitate.

Hitherto we have dealt with reactions in which the pigments produced have resulted in known fashion from the chemical combination of the reactants employed, although the distribution of these pigments in the sections of the brain is, we believe, governed by purely physical factors. But in the course of our experiments we came on another reaction in which the chemical formation of the pigment produced is less readily explained. When sections of formalin-fixed brain are soaked in solutions of alkaline sulphides, notably ammonium sulphide, the grey matter becomes pale green, probably due to the formation of ferrous and ferric sulphides. In time the white matter also becomes green. This reaction presumably depends on the initial presence of iron in the section. The colour differentiation might be due to a difference in the amount of iron in the grey and white matter, or to a difference in the chemical condition of the iron in the two parts in respect of its ability to combine with the alkaline sulphide, or to a difference in the rate of penetration of the alkaline sulphide into the two tissues. It is important to note that *all* the grey matter in the section stains uniformly, in time and in intensity. This recalls the effect in the grey matter of the reactions previously mentioned and suggests that here again physical factors are responsible.

This reaction of the formalin-fixed brain with alkaline sulphides led us to a consideration of the reaction described by Spatz (4) in 1922, which is produced by similar means but in unfixed brain. Spatz discovered that when sections of fresh (i.e. unfixed) brain are placed in a concentrated solution of ammonium hydrosulphide certain masses of grey matter speedily become dark greenish grey in colour, while other grey masses assume a lighter greenish tint after a longer interval and still other masses fail to react at all or react very doubtfully. Spatz classifies the grey masses in the central nervous system in four groups, according to their reaction to this test. In the first group are the globus pallidus and the substantia nigra, which begin to colour prominently within a few seconds. The structures in the second group react after some minutes and

include the red nucleus, the putamen, the caudate nucleus, the nucleus of Luys and the dentate nucleus of the cerebellum. Some time after these, the structures of the third group react, but with a colour distinctly less deep; these are the mammillary body, the habenular ganglion, the anterior thalamic nucleus, the cerebellar cortex and the deeper part of the cerebral cortex (the superficial part of the cerebral cortex stains very poorly if at all). Finally, the fourth group of structures react only doubtfully or not at all; they include the grey matter of the spinal cord and of the medulla oblongata (including the inferior olivary nucleus) and the geniculate bodies.

We made a series of observations with Spatz's reaction and can fully substantiate his findings as to the order of appearance and relative intensity of the coloration in the different grey masses in the fresh brain[1]. Obviously this result is very different from that obtained in any of the reactions in the fixed brain already described in this paper. Spatz believed that his reaction undoubtedly demonstrates the natural presence of iron in the reacting centres. He acknowledged the possibility that different physical conditions in different parts of the brain might affect the action of the reagent used, but decided that this possibility did not operate in the case of his reaction, and that the colour effects correspond to a varied iron content in the different grey masses such as would be shown by chemical analytical methods. He admitted that iron present in the brain in certain organic combinations (haemoglobin, for example) does not produce a reaction.

Our findings with the other colour reactions described in this paper seem to show that, in respect of them at any rate, the physical factors responsible operate equally throughout all the grey matter of the formalin-fixed brain. That these physical factors operate similarly in fresh brain, in respect of such reactions, is shown by the fact that we were able to obtain, by the prussian-blue method, a uniform staining of all grey matter in sections of fresh brain, just as in fixed material. Mainland, we find, has also obtained this reaction with fresh brain, but we do not agree with him in saying that the contrast between the grey and the white matter is then not so distinct as in fixed material; we were able to secure equal differentiation. But in all these reactions relatively large quantities of the two reacting chemicals are applied to the brain in similar ways, that is, in the form of aqueous solutions of abundant quantity. It is conceivable therefore that relatively slight differences in the degree of penetration and adsorption in the different parts of the grey matter might be masked in these reactions. In Spatz's reaction, on the other hand, only one of the reactants, the ammonium hydrosulphide, is brought into play in that fashion; the other, the iron naturally present in the brain, must be present in very much smaller amount and in very much more intimate association with the brain tissue in which the reaction is taking place. Under these conditions, it is possible that slight differences in the operation of physical factors in different

[1] It is noteworthy that the claustrum was observed by us to react in association with the cerebral cortex and not with the corpus striatum.

parts of the grey matter might exercise an appreciable effect. Thus, since Spatz's reaction and those others investigated by us take place under different conditions, it is not possible to say positively that the different result in the grey matter in Spatz's reaction is due to factors other than those physical ones which we have shown are concerned in the other reactions. But as the varied behaviour of the grey matter in Spatz's reaction is so strikingly unlike its uniform behaviour in those other reactions, it seems very likely that his reaction does to some extent at least express a different iron content in the different categories of grey matter.

Turning back now to a further consideration of the uniform staining of the grey matter produced by the action of alkaline sulphides in formalin-fixed material, it is obvious that the iron initially present in the sections cannot correspond to the natural distribution of iron which produces the very different results characteristic of Spatz's reaction. Spatz himself suggests that the formalin dissolves out the iron in the brain during the process of fixation. If that is the case, the brain would then be infiltrated with a dilute solution of iron salts and, if these were not completely washed out, the subsequent addition of an alkaline sulphide would react with the iron salt solution as do the two reactants in each of the other methods of colour differentiation we have described in this paper. A uniform coloration of all the grey matter would then be produced, and this is just what happens in this reaction. What in effect is suggested by this explanation is that the solvent action of the formalin brings about a uniform distribution of the iron throughout the grey matter of the brain, while the white matter remains relatively free from the dissolved iron for the physical reasons already discussed. When the reaction of brain with ammonium hydrosulphide is tested at intervals during the process of formalin fixation, the gradual change in result lends support to this hypothesis. Up to 2 or 3 days in 10 per cent. formalin, the characteristic Spatz reaction can still be obtained with only slight changes in detail. But later the time and depth of coloration gradually become uniform throughout the grey matter. Against this hypothesis, however, is the fact that prolonged washing (1–2 weeks) in distilled water of sections of formalin-fixed brain has no appreciable effect in diminishing the general coloration of the grey matter on the addition of ammonium hydrosulphide. We have already pointed out that in the prussian-blue and allied reactions, prolonged washing of the brain sections after immersion in the first reactant solution prevents the grey matter from colouring up on immersion in the second solution. It seems possible that the iron in the formalin-fixed brain which reacts with the sulphide is still in part at any rate in organic combination with the tissue and that the formalin alters the chemical condition of this iron, or alters the physico-chemical nature of the grey matter in such a way as to produce a uniform sulphide reaction throughout the grey matter.

It may be mentioned that the characteristic Spatz reaction can still be obtained in brain fixed in alcohol for 1–2 weeks. There is then a general quickening of the reaction times, which suggests that the fixative may cause in-

creased permeability of the grey matter generally. But alcohol does not appear to alter appreciably the factor of variability in the order and depth of staining in different parts of the grey matter; in this the action of alcohol differs markedly from that of formalin.

It will be seen that neither the sulphide reaction in fresh brain (Spatz's reaction) nor the sulphide reaction in formalin-fixed brain is comparable in all respects with those other staining reactions of the brain with which we have been chiefly concerned in this investigation. The nature of the two sulphide reactions calls for further enquiry. What will throw light on one of them will probably help to explain the other also. The extremely interesting correlations in comparative anatomy and in pathology of the groupings of the grey masses of the brain revealed by the Spatz reaction emphasise the importance of such an enquiry. Our present investigation indicates the necessity, in interpreting such reactions, of giving full consideration to the part played by purely physical factors.

SUMMARY

1. The reason for the colour differentiation of the grey and the white matter of the brain by the prussian-blue reaction has been investigated.

2. Several other similar colour reactions of the brain are described which do not involve the use of iron salts.

3. The localisation of the colour in all these reactions is shown to be governed by physical factors.

4. The action of alkaline sulphides on fresh brain (Spatz's reaction) and on formalin-fixed brain is discussed in the light of these findings.

We are much indebted to Miss W. M. Barclay-Smith for her faithful colour rendering in the illustrations of our preparations, and to Mr E. J. Weston, technical assistant in the Anatomy Department, for his help in the preparation of the specimens.

APPENDIX

The following specimen figures provide a guide to the technique employed in the reactions mentioned in the text. The reaction numbers correspond with those given in the body of the paper.

Reaction 1. *Cobalt sulphide precipitation.*

Sodium sulphide, 5 per cent. solution—3 min.
Wash in running water—5 min.
Cobalt nitrate, 1 per cent. solution—till good differentiation, then wash under tap at once.

Reaction 2. *Lead sulphide precipitation.*

Sodium sulphide, 5 per cent. solution—3 min.
Wash in running water—5 min.
Lead nitrate, 1 per cent. solution—till good differentiation, then wash under tap at once.

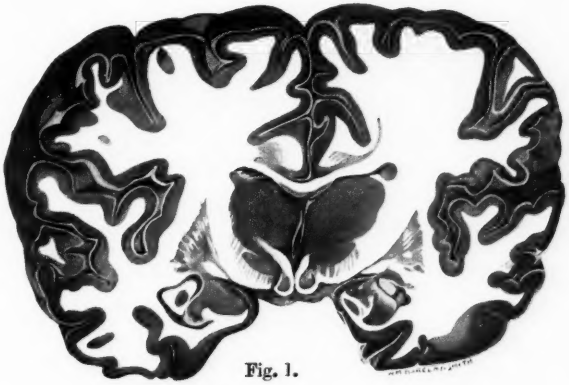

Fig. 1.

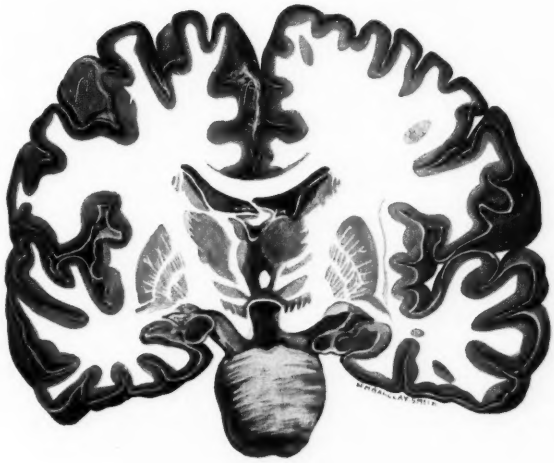

Fig. 2.

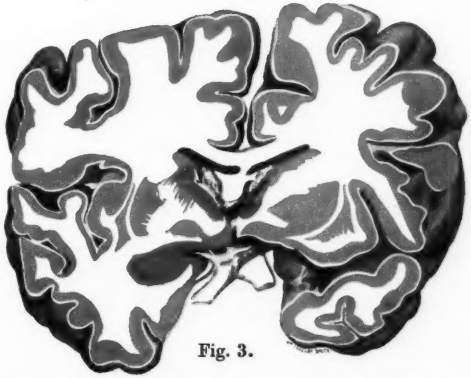

Fig. 3.

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Fig. 1.

Fig. 2.

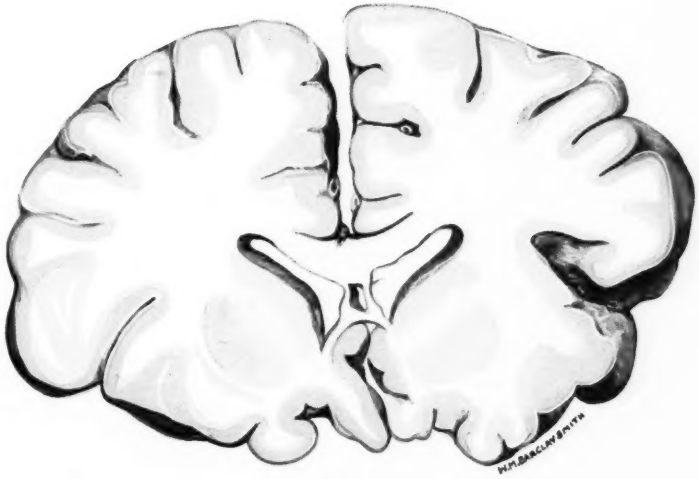

Fig. 3.

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Reaction 3. *Antimony sulphide precipitation.*

Tartar emetic, 5 per cent. solution—6 min.
Wash in running water—1 min.
Hydrogen sulphide (saturated solution of hydrogen sulphide in water plus equal volume of water)—till good differentiation, then wash under tap at once.

Reaction 4. *Lead iodide precipitation.*

Lead nitrate, 1 per cent. solution—6 min.
Wash in running water—1 min.
Potassium iodide, 5 per cent. solution—till good differentiation, then wash under tap at once.

Reaction 5. *Starch-iodine reaction.*

Soluble starch solution—24 hours.
Wash in running water—3 min.
Solution of iodine in potassium iodide—till good differentiation, then keep in 70 per cent. alcohol containing a little iodine.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Brain section stained by cobalt sulphide reaction. × $\frac{7}{12}$.
Fig. 2. Brain section stained by lead sulphide reaction. × $\frac{7}{12}$.
Fig. 3. Brain section stained by antimony sulphide reaction. × $\frac{7}{12}$.

Plate II

Fig. 1. Brain section stained by starch-iodine reaction. × $\frac{1}{1}$.
Fig. 2. Brain section stained by prussian-blue reaction. × $\frac{1}{1}$. Note coloration of area in central white matter which was abraded with a bristle brush before applying reaction.
Fig. 3. Brain section stained by lead iodide reaction. × $\frac{1}{1}$.

PREPARATION OF DRY SPECIMENS BY PARAFFIN-NAPHTHALENE IMPREGNATION

By D. M. BLAIR, F. DAVIES and E. W. McCLELLAND

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During a study of methods of differential staining of thick sections of the brain (*J. Anat.* vol. LXVI, p. 478), the difficulty arose of keeping the preparations permanently. The coloured deposit is present only in an exceedingly thin superficial part of the grey matter, so that on handling the specimens, or allowing them to rub against the sides or floor of the vessel in which they are contained, or by placing the slabs on top of each other in a vessel so that they rub against each other when the vessel is moved, the coloured layer is easily erased and the specimens spoiled. It was thought that if dry preparations of the coloured sections were made by some form of paraffin impregnation, the difficulty of handling and storage might be overcome. With this primary object in view the present work was begun.

The following was the technique at first employed. The coloured brain sections were taken through ascending alcohols to absolute alcohol. They were then immersed successively in equal parts of absolute alcohol and chloroform, in pure chloroform, in equal parts of chloroform and paraffin, and finally in melted paraffin (M.P. 56° C.). In some cases benzol or toluol was substituted for chloroform in the above process with no appreciable difference in the result. The times of immersion in the different fluids varied with the size of the slab. After immersion in paraffin (12–48 hours), the slab was suspended in the paraffin oven by cotton thread or on a long needle pushed through the specimen and supported at each end by a block of wood. The slab was then allowed to drain in the oven for 2–3 hours. In this way excess of paraffin drained off and the slab was left in its natural shape, impregnated with paraffin. It was then placed in cold water and the paraffin allowed to solidify. The result was disappointing in that the white matter of the brain had an unnatural dark brown appearance which provided a poor contrast with the coloured grey matter. This brown appearance of the white matter seemed to be due to partial translucency imparted by the paraffin. An attempt was therefore made to render the paraffin opaque by dissolving in it some substance of greater opacity. Naphthalene was found to produce the required effect.

Other coloured slabs of brain were therefore taken through the preliminary dehydrating and clearing stages as above, but were finally immersed in a solution of pure naphthalene in paraffin in the embedding oven. Naphthalene

is volatile, so that it is essential first to melt the paraffin and then add to it the solid naphthalene. Various proportions of paraffin and naphthalene were tried. The best results were obtained with a mixture of two parts by weight of naphthalene and three parts by weight of paraffin. This mixture was liquid at the normal temperature setting of the embedding oven. Smaller proportions of naphthalene do not eliminate the brownish appearance of the white matter of the brain and greater proportions of naphthalene give an artificial whiteness to the entire specimen. In this way thick sections of brain were prepared which retained the appearance, colour and shape which they had when in the form of wet specimens and they could now be handled freely and stored readily without damage. Such specimens are of great use for teaching purposes, since they can be passed safely round a class and the specimens do not need replacement at comparatively short intervals. Painting the surface of the solid slabs with copal varnish acts as a further protection and tends to prevent any loss of naphthalene that might occur by evaporation.

This method of embedding was then applied to organs removed from the dissecting room cadaver (formalinised) with good results. With paraffin-naphthalene impregnation the original appearance and colour of the organs is much more faithfully preserved than when paraffin alone is used for embedding.

PREPARATION OF HISTOLOGICAL SECTIONS OF THE DRY SPECIMENS

The paraffin-naphthalene method of preparation of dry specimens has the added advantage that it is possible at any time subsequently to obtain good histological sections. About four months after making paraffin-napthalene preparations of the kidney, suprarenal and ovary of a full-time foetus and of a slab of adult brain, a small cube (about 1 cm. cube) was cut from each with a sharp knife. The cubes were placed in melted paraffin for about two hours, blocks were made and sections cut and stained in the usual manner. The tissue elements stained well and showed no appreciable shrinkage or distortion as compared with preparations made in the usual way.

The method of paraffin-naphthalene impregnation therefore, in addition to providing naked eye specimens of natural appearance which can be safely handled, has the advantage also that good histological preparations can be made at any time. The method should thus be of especial use for the preservation of pathological material.

We wish to express our thanks to Mr E. J. Weston, technical assistant in the Anatomy Department, for help in the preparation of the specimens and histological sections.

SYMPATHETIC ELEMENTS IN THE CRANIAL AND SPINAL GANGLIA

By F. KISS

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I. MATERIAL AND METHOD[1]

This work is based on an anatomical examination of the cranial ganglia and their connections in all classes of vertebrates. The greatest variations were found in Mammals. In the present paper I shall refer only to such of my anatomical results as are essential for the interpretation of the histological part of my research.

Fresh human ganglia and those of different Mammals were examined by a new method, consisting essentially of subjecting series of sections to osmic acid for prolonged periods:

(1) Fixation in 10 per cent. formol (4 per cent. formaldehyde) 24 hours.

(2) Washing in distilled water 24 hours, shaking the water three or four times.

(3) 1 per cent. osmic acid 5–8 days. The quantity of the solution must be adequate. The glass must always be thoroughly and carefully cleansed, otherwise the osmic acid will be reduced. The vessel must be slowly turned at least three to four times daily, otherwise the material will not be adequately exposed to the influence of the osmic acid.

(4) Wash in distilled water 12–24 hours. The water must be slowly shaken with the material three or four times. Insufficiently washed materials contain osmic acid in the intercellular tissue, which will be reduced in the alcohol and produce a black precipitate.

(5) Alcohol series (30–50–70–96 per cent., etc.).

(6) Embedding into celloidin-paraffin (Apáthy's method). Careful double (celloidin-paraffin) embedding prevents shrinkage. Shrunken cells are useless for this study, both form and colour are influenced by shrinkage. I used for dehydration with good results 3–5 per cent. carbolic acid upward to the 96 per cent. alcohol (modified method of Barta).

(7) Cut complete series of sections.

(8) Some of the slides to be counterstained with eosin.

[1] The research was done in the Anatomical Department of University College, London, and in the Prosectorium of the Zoological Gardens (London), and I wish to express my thanks to Prof. Elliot Smith and Dr Zuckerman, as well as to Mr F. Pittock who made the microphotographs.

II. DESCRIPTION OF THE PREPARATIONS

By the prolonged osmic acid method two types of nerve cells can be differentiated in the cranial ganglia. Form, size and coloration of the two types are clearly distinct.

Type I (fig. 1, *a*) is apolar (or unipolar), the cell body (protoplasm) is pale with very fine granules; the size (diameter) is different (smaller or larger), the nucleus is uncoloured.

Type II (fig. 1, *b*) is always multipolar, the cell body (protoplasm) is dark (black), the granules are larger than in type I. Even the small forms of type I can easily be differentiated from type II.

To determine the two types I examined by my method different sensory and sympathetic ganglia. In human spinal ganglia (fig. 2) I found a great majority of type I (*a*) and scattered amongst the large pale cells single ones of type II. About the same proportion is found in the Gasserian ganglion of Man and Mammals (fig. 3).

In the sympathetic ganglia of Man (fig. 4) and different Mammals (monkeys and pigs), I found uniformly only small, dark, multipolar cells (type II). There were many unmyelinated bundles and fibres originating from ganglia with cells of type II. In my comparative anatomical study I often found that sensory and sympathetic nerves meet in the same ganglion (figs. 9 and 10). These three circumstances led me to the conclusion that the cells of type I are sensory elements, and that those of type II are sympathetic.

The different cranial ganglia are composed of different combinations of these two elements.

(1) Ciliary ganglion (fig. 1)

Cells of the two types are represented by about the same numbers. The cells of type I (sensory) generally lie close to one another, those of type II (sympathetic) are in this as in the other ganglia usually scattered.

The proportion of the two types may be different in animals. In *Cercopithecus* (fig. 5) for instance the dark (sympathetic) cells form the great majority. I also found in the macaque that one part of the ciliary ganglion contains more sensory, the other part more sympathetic cells. It may be that new embryological investigations will explain these proportional differences[1].

I examined carefully a complete serial section of the roots and branches of the ciliary ganglion in Man and other Mammals. At the same time I studied the topography of this ganglion in different vertebrates.

(*a*) *Relation of the ciliary ganglion to the third nerve*

Like previous authors I found that the ciliary ganglion is situated in the trunk of the third nerve in Reptiles, Birds, and in some Mammals, for example, *Chiromys madagascarensis* (aye-aye), sloth bear (*Melursus ursinus* Shaw), etc.

[1] So far as I know embryological investigations hitherto have not taken cognisance of the two types of the cells described above.

The ganglion generally receives its largest (thickest) root from the third nerve, as in Man. I examined this connection on fresh human materials in serial sections, stained with osmic acid, and found that the trunk of the third nerve contains more thinly myelinated fibres, among the heavily myelinated fibres, than the fourth or sixth nerve. I also found that the ciliary ganglion receives, through the so-called short root exclusively, thinly myelinated fibres from the third nerve.

In my previous investigations[1] I confirmed Gaskell's observation that the thinly myelinated fibres of the anterior spinal roots are all pre-ganglionic, and that their number depends upon the size of the white ramus communicans, by which a spinal nerve is connected with a sympathetic ganglion. The larger number of the thinly myelinated fibres in the third nerve depends upon the connection with the ciliary ganglion. It seems that the sympathetic (dark) cells of the ciliary ganglion are in the same connection with the third nerve, as for example a thoracic sympathetic ganglion with the anterior (motor) spinal root of the same segment. It means also, that the short (or motor) root of the ciliary ganglion contains pre-ganglionic fibres. There is no morphological reason to differentiate this connection from the ordinary sympathetic system and call it "parasympathetic." Cells, pre-ganglionic fibres and connection to a motor (third) nerve have the usual sympathetic characters.

(*b*) *Relation of the ciliary ganglion to the trigeminus and to the sympathetic* (*carotid*) *plexus*

The long (sensory) root of the ciliary ganglion consists for the most part of thinly myelinated, with a minority of heavily myelinated, fibres. This agrees with a posterior spinal root. It seems also that the pale (round) cells of the human ciliary ganglion are in the same relation through the long root to the trigeminus, as a spinal ganglion to the posterior root. As I have already demonstrated (fig. 2) the human spinal ganglion also contains dark, multipolar (sympathetic) cells. The real function and connections of these cells is not yet known. Their pre-ganglionic fibres are probably in the posterior spinal roots. As efferent fibres, they should degenerate after section of the root in a direction opposite to that of the sensory (afferent) fibres. Hitherto these fibres have been ignored in the numerous experiments on posterior spinal roots.

The sympathetic root of the human ciliary ganglion contains for the most part unmyelinated fibres associated with a small number of finely and a few heavily myelinated fibres. I found in some cases two or three fine (microscopic) roots of this kind joined to the long root of the ganglion. This is the reason why sometimes, by anatomical dissection, the sympathetic root is not found at all.

[1] Kiss and Mihálik. "Ueber die Zusammensetzung der periph. Nerven und den Zusammenhang zwischen Morphologie und Funktion der periph. Nervenfasern." *Zeitschr. f. Anat. u. Entwicklungsgesch.* Bd. LXXXVIII, H. 2, 1928.

Kiss. "The relationship between vagus and sympathetic in the Vertebrates." *J. Anat.* vol. LXVI, p. 153, 1931.

The sympathetic root connects the ciliary ganglion to the fibres and small ganglia of the carotid plexus, and we can regard it as an ordinary inter-ganglionic part of the cranial sympathetic.

(*c*) *Branches of the ciliary ganglion* (*ciliary nerves*)

Examining the branches of the ciliary ganglion and its roots in series sections I found in the human ciliary nerves only two kinds of fibres: (1) a great majority of thinly myelinated and (2) a minority of unmyelinated fibres (fig. 6). The unmyelinated fibres form in *Macacus* unstained groups amongst the myelinated fibres (in transverse sections) or occupy (in Man) one side of the nerve (fig. 6, *c*, *c*). The ciliary ganglion also sends unmyelinated fibres to the trunk of the third nerve, which are distributed with the branches of this nerve.

On the basis of my previous investigations[1] I regard the thinly myelinated fibres as sensory, the unmyelinated as ordinary sympathetic (motor and secretory) fibres.

It seems that the sensory and sympathetic cells are, in the cranial ganglia, in a special combination which enables them to transfer sensory impulses directly to sympathetic cells and fibres (for example, secretion of tears through irritation of the conjunctiva, etc.).

(2) Spheno-palatine ganglion

I found in the human spheno-palatine ganglion two kinds of cells: a majority of dark, multipolar (fig. 7, *b*) and a minority of pale, round cells (fig. 7, *a*). The latter type is mostly of the smaller form. I found large unmyelinated nerves in the ganglion (fig. 7, *c*), and many thinly myelinated fibres between the cells.

The same ganglion of *Macacus* (fig. 8) is wholly composed of dark, multipolar cells. If these cells alone form the ganglion, they are dispersed in its stroma. The pale, round sensory cells, usually lay close to one another (fig. 1, *a*).

In my comparative anatomical study I found in different animals (figs. 9, 10) that the sympathetic trunk is continued above to the superior cervical ganglion, directly into the spheno-palatine ganglion. Very often in various animals this is enclosed in the palatine and nasal branches of the maxillary nerve. The ganglion is in such cases multiple. Figs. 9 and 10 show anatomically the meeting of sensory and sympathetic nerves in the ganglion.

The sympathetic trunk, which in the llama and in the tree kangaroo connects the superior cervical ganglion with the spheno-palatine ganglion, is represented in Man by the major superficial petrosal nerve (fig. 11, I). This nerve contains myelinated (*d*) and unmyelinated (*c*) fibres. The unmyelinated fibres connect the geniculate ganglion (fig. 16) with the spheno-palatine ganglion. The major superficial petrosal and the Vidian nerve are homologous to an inter-ganglionic part of the great sympathetic. The Vidian nerve also receives

[1] *Loc. cit.*

many unmyelinated fibres through the deep petrosal nerves (from the carotid plexus).

(3) Otic ganglion

This is one of the largest cranial ganglion. It is enclosed in the perineurium of the mandibular nerve, and its diameter is larger than the transverse section of the nerve (fig. 12).

The human otic ganglion is composed exclusively of small, dark, multipolar (sympathetic) cells. Many unmyelinated nerves are connected with this ganglion (fig. 13, *c*, *c*). They proceed to different organs, or join different branches of the mandibular nerve. The minor superficial petrosal nerve (fig. 11, II) contains myelinated and unmyelinated fibres. It represents an inter-ganglionic connection between the otic and the petrous ganglion of the ninth nerve (fig. 19), which also contains dark (sympathetic) cells.

The otic ganglion of the cat (fig. 14) is mixed; the majority of the cells belong to the round, pale (sensory) type.

In other animals the ganglion is subject to a wide range of variation. It seems that the proportions of sensory and sympathetic elements may be very different in the different families. Experimenters on living animals should not neglect these differences.

(4) Submaxillary ganglion

I always found that the human submaxillary ganglion (fig. 15) contains exclusively dark, multipolar (sympathetic) cells. These are scattered in the stroma, as in an ordinary sympathetic ganglion. The human submaxillary ganglion is in fact an ordinary local ganglion.

(5) Geniculate ganglion

In the human geniculate ganglion (fig. 16) only dark, multipolar cells were found. These cells lay separated in the stroma. There are a few cells in fig. 16, which look rather pale than dark, but these are multipolar too.

This ganglion of *Cercopithecus* (fig. 17) contains both dark and pale cells. The cells generally lay closer than in Man.

(6) Ganglia of the ninth nerve

The jugular ganglion of the ninth nerve in Man contains, mostly multipolar, dark (sympathetic) cells (fig. 18). The petrous ganglion of *Nasua* (fig. 19) contains a majority of round, pale (sensory) cells. The two ganglia of the ninth nerve are often united in different animals, or one of these ganglia is represented only by cells in the trunk of the nerve without any gangliform enlargement.

(7) The ganglia of the tenth nerve

The ganglion of the root (jugular ganglion) of the human vagus nerve (fig. 20) contains a great majority of multipolar, dark (sympathetic) cells which lay scattered in the stroma. The kind of fibres which connect this ganglion to

other nerves (facial and spinal accessory), and to other ganglia (petrous and superior ganglion of the sympathetic), I have not yet investigated.

I found a very interesting section in *Nasua* (fig. 21). The preparation shows the lower end of the jugular ganglion (with sympathetic and sensory cells). Close to the ganglion is a nerve with mostly unmyelinated fibres (*c*). The spinal accessory has two parts, one of them (d_1), which lies close to the vagus, is composed mostly of thinly myelinated fibres. This is the internal branch of this nerve. The other portion (d_2) is composed principally of heavily myelinated fibres. This is the external (motor) branch of this nerve.

The ganglion of the trunk (ganglion nodosum) is different from the previous ganglion. It is more like a spinal ganglion (fig. 22). The round, pale cells (*a*), which form the great majority, lie close to one another. The single, multipolar, dark cells (*b*) are dispersed among the pale cells.

The ganglion nodosum of *Cercopithecus* (fig. 23) agrees with that of Man. The same ganglion of the pig (fig. 24) very distinctly shows the two types (*a* and *b*) of cells. The vagus, therefore, receives unmyelinated fibres not only through the anastomoses with the cervical sympathetic, as I claimed in my previous paper on this subject ("Le rapport entre le pneumog. et le grand symp." 1931), but also from the sympathetic cells of its own ganglia.

III. DISCUSSION OF THE LITERATURE

The multipolar, dark cells of some cranial and spinal ganglia are mentioned in the literature under different names. Some writers described them as pathological or senile forms of the ganglionic cells, others regarded them as argyrophyl cells. They represent one of the eleven groups of Dogiel. Some of the authors saw single multipolar cells and described them as sympathetic, but no author and no method described and demonstrated them as systematically and distinctly as I have attempted to do in the present paper.

Tangential sections of type I or type II may give the appearance of intermediate forms, but serial sections always prove that these forms belong either to type I or type II. This is the explanation of the intermediate cells of some authors.

Most writers describe the cranial ganglia as homologous to the spinal ganglia, but several authors found single multipolar cells in this or in that ganglion. Thus G. Retzius (1880), His (1888–90), F. Martin (1890) and M. Lenhossék (1893) described the geniculate ganglion as a real spinal ganglion of the intermedius nerve. Gaskell (1889) thought that the ganglion nodosum is a sympathetic ganglion; Van Gehuchten (1897) regarded the same ganglion as analogous to the spinal ganglion. In this communication it has been shown that we cannot generalise as to the nature of any cranial ganglion.

Disse (1893) found multipolar cells in the spinal ganglia of frog embryos. M. Lenhossék (1893) found multipolar cells in the spinal ganglia of chick embryos. He described the processes of these cells as dendrites. Lenhossék also

examined the spinal and cranial ganglia of Mammals, but he could not find multipolar cells in them. Van Gehuchten agreed with Lenhossék. Ramón y Cajal (1893) also saw multipolar cells in the spinal ganglia of chick embryos. He described the processes as short dendrites which later disappear through a regressive metamorphosis. Retzius (1894) described multipolar cells in the spinal ganglia of the rat and of snakes (*Ophidia*). Kölliker (1896) illustrates in his manual a multipolar cell from the Gasserian ganglion of the calf. Spirlas (1896) found, by means of Golgi's method, multipolar cells in the spinal ganglion of Mammals. Dogiel (1896) described multipolar cells in the spinal and Gasserian ganglia of the dog, cat, rabbit and guinea-pig. Huber (1896), described the same cells in the tortoise (*Chelydra*) and in chickens. Van Gehuchten (1897) found multipolar cells in the 10 cm. embryos of the snake (*Tropidonotus natrix*); Dogiel (1898) in the spinal ganglia of Man and other Mammals, Retzius (1898) in embryos of the chicken, rabbit and snake. Barratt (1898) regarded the two ganglia of the vagus as similar to one another ("The cells of the two ganglia were not obviously distinct in character," p. 426). Cannieu (1898) described multipolar cells in the cranial ganglia of different mammals.

Herrick (1899) illustrates in his monograph how the sympathetic crosses over the cranial nerves in fishes, which I also described in the cod (*Gadus*). Comparative anatomical studies like these show distinctly that the cranial part of the sympathetic is in immediate connection with many cranial nerves and ganglia. This relation is clearly illustrated in a teleostean fish (*Uranoscopus scaber*) by J. Z. Young (1931). Holmgren (1899), found multipolar cells in the spinal ganglia of another teleostean (*Lophius piscator.*), Levi (1903, 1906) in tortoises and selachians. Ramón y Cajal (1906) again described multipolar cells in the sensory ganglia of adult Man, donkey, horse and cattle, without the physiological characteristics of these cells. Nageotte (1907) found the same cells in transplanted spinal ganglia of the rabbit; he regarded them as transformed unipolar cells. Lenhossék (1907) found, in the human spinal ganglia, cells which had multiple axons (Cajal's method). Marinesco, Parhon and Goldstein (1908) found by the silver method of Cajal three different types of multipolar cells in the ciliary ganglion of Man, monkey and dog. The authors suggested the sympathetic nature of this ganglion. Dogiel (1908) regarded the multipolar cells in the spinal ganglia as embryonic forms, which later replace the old cells. Takeda (1924) found, in the Gasserian ganglion of cattle, several multipolar cells. He regarded the processes partly as dendrites, partly as axons.

This list of investigations reveals how individuals of the numerous multipolar (dark) cells emerged through the silver and methylene-blue methods, but none of the above-mentioned authors by these methods, and on the basis of isolated, and not serial, preparations was able to obtain a general view of the multipolar (sympathetic) cells in the cranial and spinal ganglia.

Riquier (1914) found that the otic ganglion of cattle and of Man is similar to a sympathetic ganglion.

It is more interesting to follow some clinical and experimental observations in recent literature.

Stewart and Lambert (1930) found that pain or circulatory and secretory troubles of the nasal cavity are dependent upon the spheno-palatine ganglion. Anaesthesia, or extirpation of this ganglion, helped in many cases. It was also observed that the disturbed function of the spheno-palatine ganglion was in relation with sympathetic diseases of other organs, for example, the stomach. These observations suggest the sensory and sympathetic characters of this ganglion.

Yungoro Takagi (1931) cut the posterior spinal roots in cats. Like other authors he obtained very contradictory results. He often found that many fibres were not degenerated in the central portion of the operated posterior root. Like previous writers he came to the conclusion that the posterior root contains efferent fibres. These efferent fibres are, according to my investigations, most probably the pre-ganglionic fibres of the sympathetic cells in the spinal ganglia.

Ken Kuré (1928–31) published, with his fellow-workers, a series of experiments on dogs. They made radicotomies on the posterior spinal roots, and found numerous thinly myelinated fibres in the central stump without degeneration. These are called by Kuré "spinal parasympathetic nerves." The authors do not mention any sympathetic cells in the spinal ganglia. The undegenerated fibres are probably simple pre-ganglionic fibres. There is no reason for the supposition of a "spinal parasympathetic" system. Kuré and his fellow-workers impute to this system tonic, trophic, vaso-dilatory, stimulating and vaso-constrictory functions. The suppositions of these authors only increase the great confusion about the morphology and physiology of the vegetative nerves.

S. W. Ranson and H. K. Davenport (1931) published a paper on the "Sensory unmyelinated fibres in the spinal nerves." They extirpated in cats the lumbar and sacral part of the sympathetic trunk. Thirty-nine to eighty-four days after the operations they still found unmyelinated fibres in the saphenous nerve and in a motor branch of the femoral nerve. They call them "sensory unmyelinated fibres." The authors do not mention the sympathetic cells in the spinal ganglia; the unmyelinated fibres (of Ranson and Davenport) are probably the simple post-ganglionic fibres of the sympathetic cells in the spinal ganglia. The supposition that the sympathetic has some (sensory) function, other than effective (motor or secretory), only adds another great element of confusion to the literature of the vegetative nervous system.

F. Rossi (1931) found in human embryos multipolar cells in the central portion of the rami communicantes of the lumbar and sacral region. He regards them as small sympathetic ganglia and describes them as different from the "microsympathetic, hypospinal ganglia" of Marinesco and Minea (1908).

It is a common phenomenon to find in the cranial ganglia that multipolar, dark (sympathetic) cells are located in the central portion of the branches, which contain sympathetic (unmyelinated) fibres (for example ciliary nerves). The ganglia described by Rossi and those described by Marinesco and Minea

are the same groups of cells and they are simple sympathetic cells of the spinal ganglia exposed in the rami communicantes. H. Streckfuss claimed in a recent paper (1931) that the greater splanchnic nerve contains in its roots and trunk many small ganglia. He therefore calls this nerve a splanchnic ganglionated cord.

The sympathetic cells are not located exclusively in the ganglionated cord, but partly in their embryonal places in the cranial and spinal ganglia. There is, as yet, no morphological evidence that all the local ganglia of the organs are not common sympathetic elements.

IV. SUMMARY

1. The prolonged osmic acid method differentiates two types of cells in the cranial and spinal ganglia.

2. Cytological characters, histological circumstances (unmyelinated branches) and comparative anatomical evidence prove that the multipolar, dark, small cells (type II) are sympathetic, the round, pale and generally larger cells (type I) are sensory.

3. The human ciliary, spheno-palatine, Gasserian and both ganglia of the ninth and tenth nerves are composed of sensory and sympathetic cells.

The otic, submaxillary and geniculate ganglia in Man contain only sympathetic cells.

4. The proportions of the sensory and sympathetic cells in the same ganglion are also variable in the different Mammals.

5. Human spinal ganglia also contain a few sympathetic cells.

6. Sympathetic cells emigrate, not only between the second dorsal and fourth lumbar segments (Gaskell), but also through the cranial ganglia.

7. There is no morphological foundation for the supposition of a cranial "parasympathetic system." The cranial parasympathetic of literature is a part of the cranial sympathetic.

8. The ganglia of the cranial sympathetic are in the same relation to the cranial nerves (through communicating branches) as the ganglia of the great sympathetic to the spinal nerves.

9. The terminal member of the cranial sympathetic is the ciliary ganglion. The ganglia of the cranial sympathetic are linked together through interganglionic fibres, like the ganglionated cord.

10. The mixed (sensory and sympathetic) ganglia may be the transformators of sensory (centripetal) affects into vegetative (centrifugal) effects; for example, irritation of the cornea, secretion of the lachrymal gland, etc.

11. The effective (centrifugal) fibres of the posterior spinal roots, "spinal parasympathetic" of Ken Kuré, are at least partly pre-ganglionic fibres of the sympathetic cells in the spinal ganglia. The "sensory unmyelinated fibres" of Ranson and Davenport are simple post-ganglionic fibres of the same cells.

12. My investigations afford no evidence for the existence of a "cranial parasympathetic."

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EXPLANATION OF PLATES

(Microphotos of the same magnification.)

Plate I

Fig. 1. Ciliary ganglion of Man.
Fig. 2. Fourth spinal thoracic ganglion of Man.
Fig. 3. Gasserian ganglion of Man.
Fig. 4. Thoracal sympathetic ganglion of Man.
Fig. 5. Ciliary ganglion of *Cercopithecus*.
Fig. 6. Ciliary nerves of Man. I = transverse, II = longitudinal section.
Fig. 7. Spheno-palatine ganglion of Man.
Fig. 8. Spheno-palatine ganglion of *Macaca*.

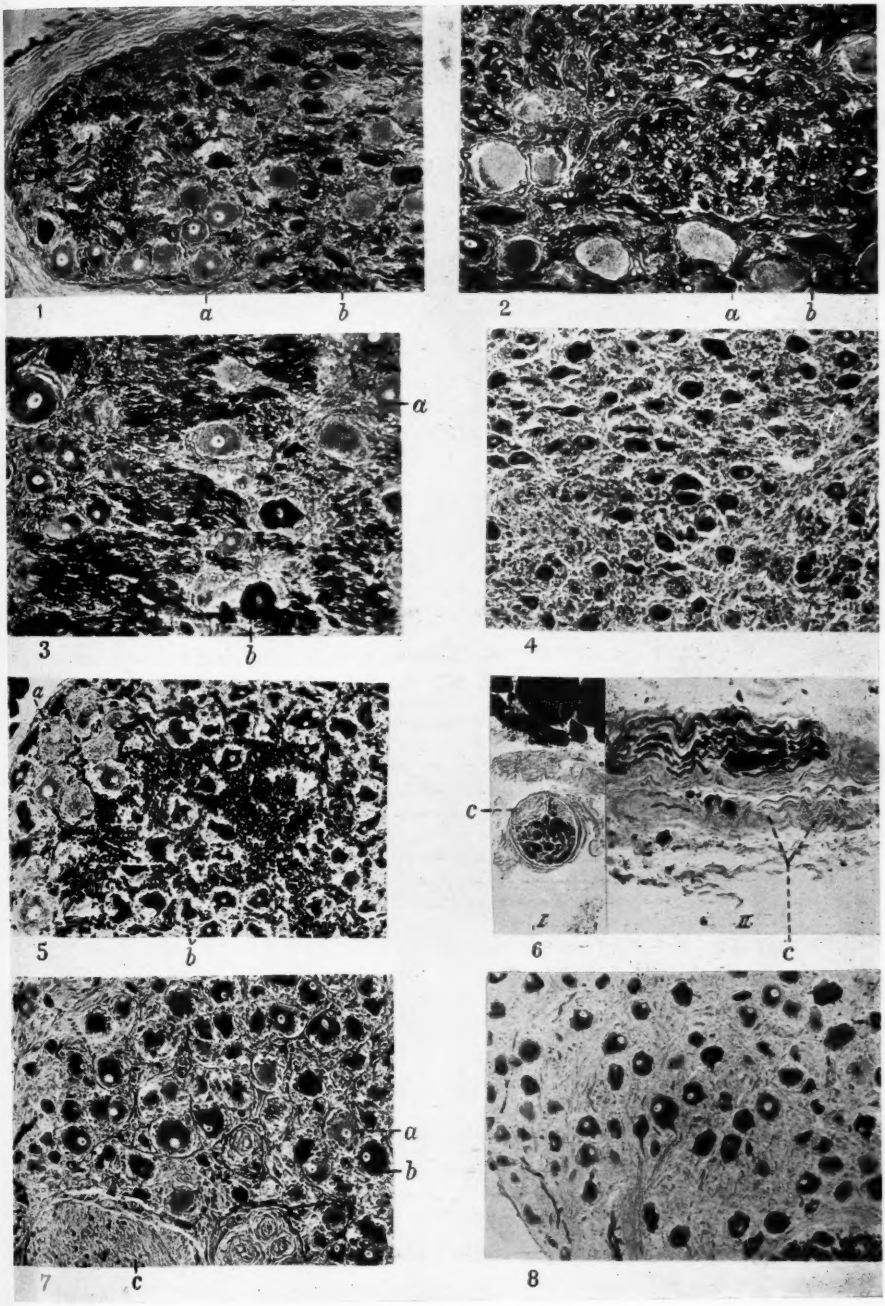
Plate II

Fig. 9. Llama: roots of the spheno-palatine ganglion.
Fig. 10. Tree Kangaroo: roots of the spheno-palatine ganglion.
Fig. 11. Superficial petrosal major (I) and minor (II) nerves of Man.
Fig. 12. Otic ganglion of Man (transverse section with mandibular nerve).
Fig. 13. Otic ganglion of Man.
Fig. 14. Otic ganglion of cat.
Fig. 15. Submaxillary ganglion of Man.
Fig. 16. Geniculate ganglion of Man.

Plate III

Fig. 17. Geniculate ganglion of *Cercopithecus*.
Fig. 18. Jugular ganglion of the ninth nerve in Man.
Fig. 19. Petrous ganglion of the ninth nerve in *Nasua*.
Fig. 20. Jugular ganglion of the tenth nerve in Man.
Fig. 21. Jugular ganglion of the tenth nerve in *Nasua*.
Fig. 22. Ganglion nodosum vagi of Man.
Fig. 23. Ganglion nodosum vagi of *Cercopithecus*.
Fig. 24. Ganglion nodosum vagi of pig (*Sus scrof. dom.*).

Letters on the figures:
a = sensory, *b* = sympathetic cells, *c* = unmyelinated, *d* = myelinated fibres.



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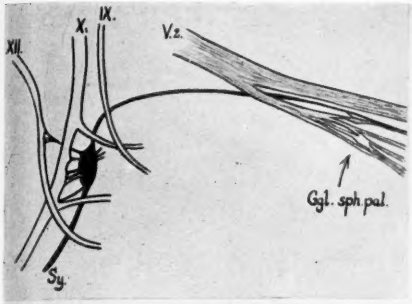

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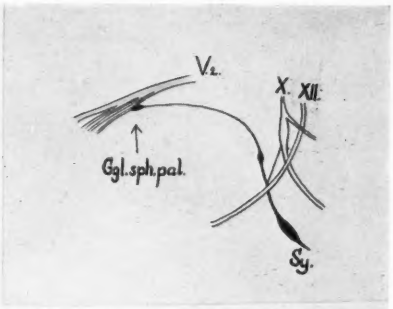

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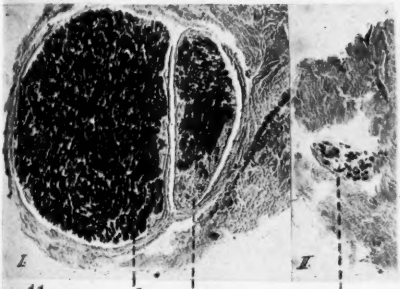

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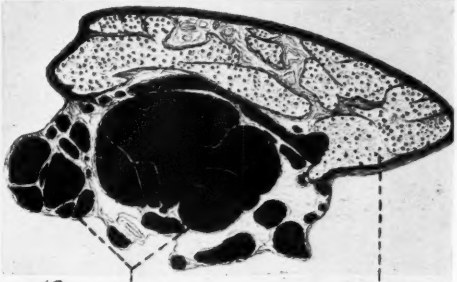

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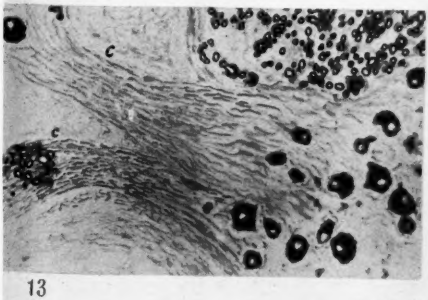

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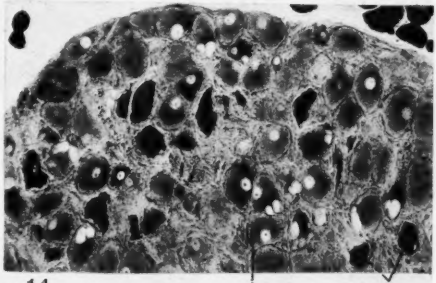

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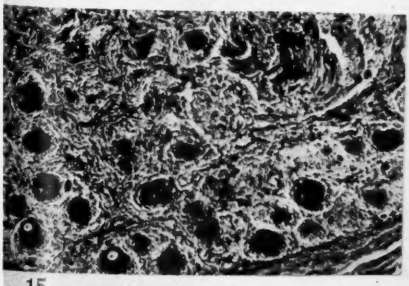

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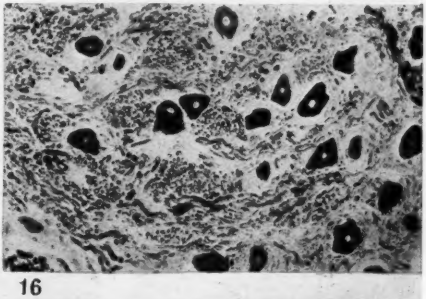

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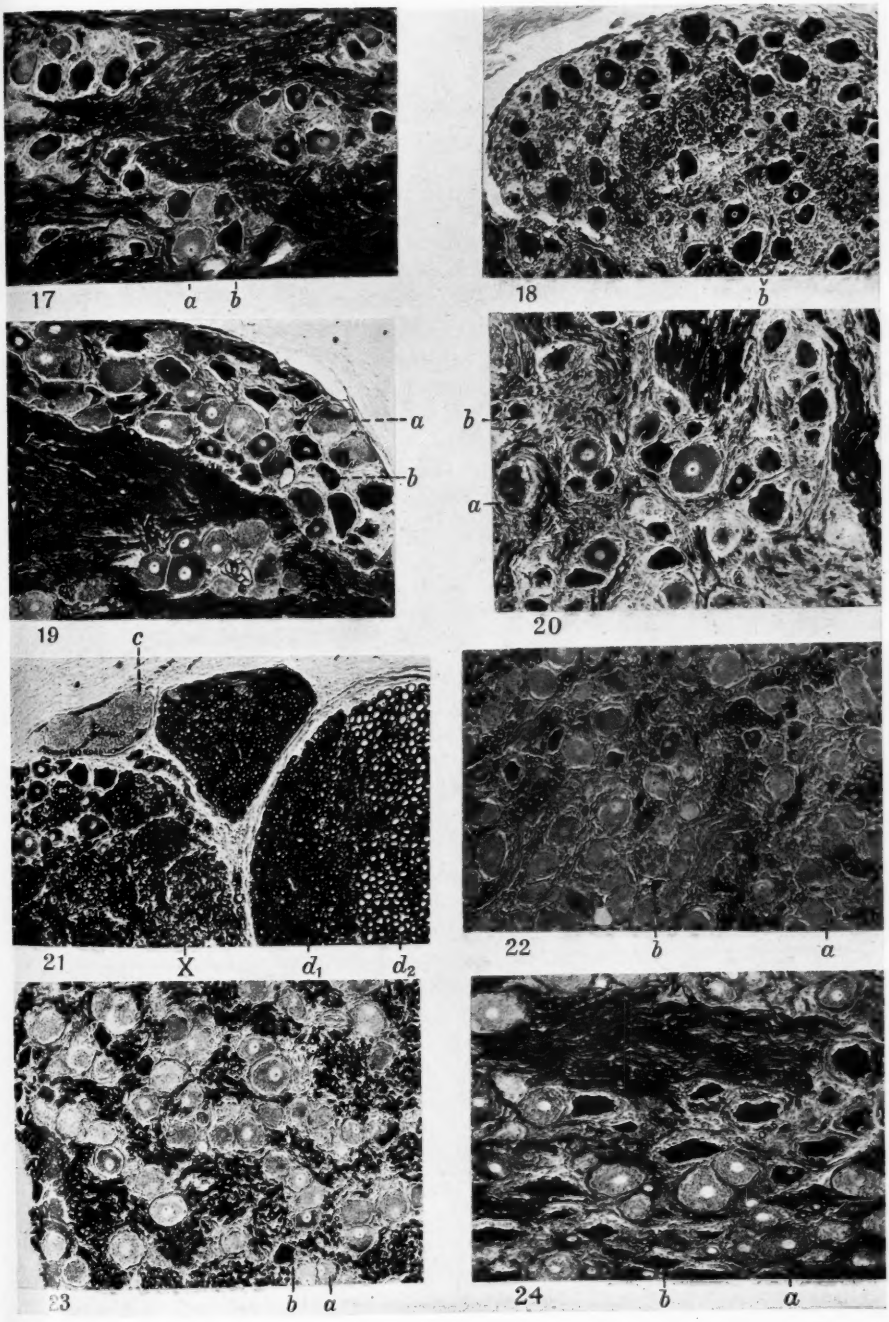

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OBSERVATIONS ON THE DISTRIBUTION OF THE ARTERIAL BRANCHES OF THE PERIPHERAL NERVES

By A. E. COATES

Anatomical Department, University of Melbourne

The present research was undertaken in order to determine the exact distribution of the arterial branches of the peripheral nerves, since such knowledge must be of value in the interpretation of symptoms of peripheral vascular derangement and also in the planning of arterial operations.

The writer has been interested for some years in the problem of the arterial nerve supply and has frequently performed the operation of so-called "Peri-arterial Sympathectomy." Since it is obvious that the latter operation gives variable results in the hands of different workers, it was deemed advisable to investigate the precise anatomy of the vascular nerves. The present paper deals only with the branches of peripheral nerves passing to large arteries of the limbs. The material investigated consisted partly of cadavera in the anatomical department and partly of limbs amputated by the writer for various surgical conditions. Observations were also made on patients subjected to peri-arterial sympathectomy in order to establish the relation of the peripheral nerve branches to the arteries in question.

In the cadaver the main nerves and arteries were dissected from the limb *en bloc*, and the arterial branches of the nerves were then teased out; sections of these nerves were cut, stained and examined microscopically. The accompanying photographs illustrate the origin of the branches, and the micro-photographs show that there are many medullated fibres of large size found in the twigs to the arteries. The dissection of the small arterial twigs of the digital nerves was found impracticable in the material available, so that the larger branches in the arm and forearm, pelvis, thigh and leg are described. The results obtained in this series do not quite coincide with those of Kramer and Todd (1) as will be seen from the text, but more closely resemble the description given by Goering in 1834 (quoted by Kuntz (2)). That the nerves in question enter the adventitia of the artery can be seen from the photograph of a branch of the saphenous nerve supplying the femoral artery in Hunter's canal (fig. 1).

The following is a description of the nerves found in this series:

The brachial artery receives two branches in some cases and one in others from the accompanying median nerve. These branches leave the nerve at an acute angle and pass into the adventitia of the artery after running a variable distance along the vessel. The first appears to join the artery in the upper third and the second in the lower third of the arm. Fig. 5 illustrates these nerves,

and the accompanying microphotograph shows that medullated fibres are present.

In one case the only vascular branch to the brachial artery appeared to arise from the musculo-cutaneous nerve as stated by Kramer. Dissection showed, however, that the nerve actually arose from the lateral fasciculus of the brachial flexus which was abnormal in that it merged into the median nerve before splitting off the musculo-cutaneous nerve (see fig. 7).

In five cases dissected no evidence was found of any branch from the musculo-cutaneous nerve passing to the brachial artery. The variations found suggest that it is not impossible for such an arrangement to be quite common. In the forearm, the ulnar artery receives a rich nerve supply.

In fifteen specimens examined, a branch of the ulnar nerve in the upper third of the forearm passed on to the ulnar artery and appeared to run along the artery a considerable distance before losing itself in the arterial sheath (see fig. 8).

In two cases the ulnar artery received two branches from the ulnar nerve, one in the upper third and the other in the lower third of the forearm (see fig. 9). It will be observed from the microphotographs of both the median and ulnar branches that even when stained with osmic acid, there are two types of nerve fibre, large medullated, and small non-medullated or partly medullated fibres. The radial artery was found in three cases to receive a branch from the median nerve in the region of the wrist. No branches from the radial nerve were found to supply the artery in any part of the forearm.

The common interosseous artery received a branch from the median nerve. This branch, which is large, joins the artery just distal to its origin from the ulnar artery. Fig. 8 illustrates this point and also shows the arterial supply of the median nerve.

For the upper extremity the nerve supply to arteries is as follows:

Brachial artery receives one or two branches from the median nerve.

Ulnar artery receives a branch of the ulnar nerve and the radial artery receives a branch of the median nerve.

The common interosseous artery receives a branch from the median nerve.

These nerve filaments contain both medullated and non-medullated nerve fibres (see fig. 6).

In the lower extremity a branch of the femoral nerve passes to the external iliac artery in the pelvis. The femoral nerve gives off two branches to the common femoral artery in the upper third of the thigh. The saphenous nerve supplies a constant branch to the femoral artery at the lower end of Hunter's canal.

The popliteal artery is supplied by a small ramus or two from the tibial nerve in the popliteal space (see fig. 4).

The posterior tibial artery receives several branches from the tibial nerve (two or three), the first joining the artery just below its origin and the last in the neighbourhood of the medial malleolus.

Fig. 1. Branch of saphenous nerve entering sheath of femoral artery in Hunter's canal.

Fig. 2. Branch of saphenous nerve passing to femoral artery—two-thirds natural size.

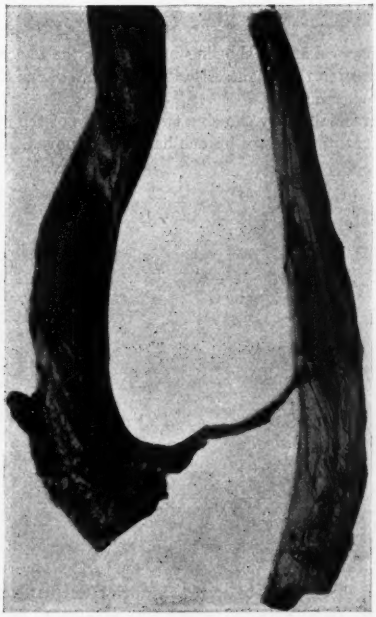

Fig. 3. Branch of femoral nerve passing to the external iliac artery 1 inch proximal to Poupart's ligament.

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Fig. 4.

Fig. 5.

Fig. 4. Dissection of the femoral artery showing branches of the femoral and saphenous nerves joining the femoral artery in the thigh. Also two branches of the tibial nerve passing to the popliteal in the popliteal space.

Fig. 5. Dissection of the brachial artery showing two branches of the median nerve entering the sheath. A small segment of the lower branch has been removed.

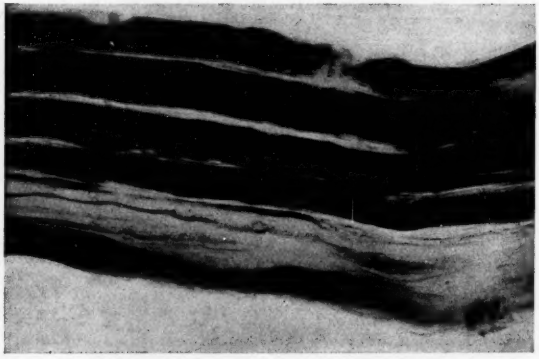

Fig. 6. Microphotograph of the segment of arterial branch of median nerve seen in fig. 5. Stained with osmic acid. Note the presence of medullated nerve fibres.

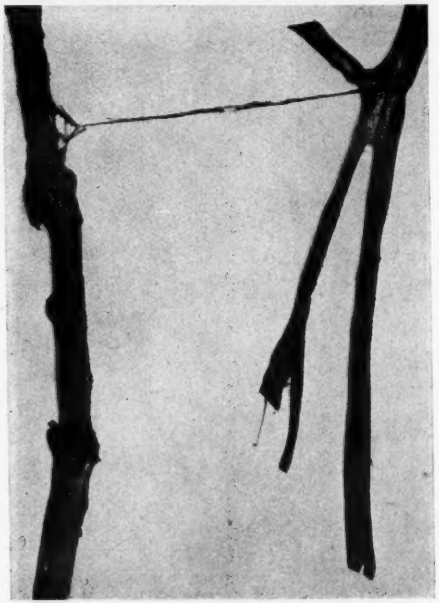

Fig. 7. Branch of lateral fasciculus of brachial plexus passing to brachial artery.

Fig. 8.

Fig. 9.

Fig. 8. Dissection of the arteries and nerves of the forearm. The brachial artery seen in fig. 5 is here dividing into radial and ulnar arteries. The radial nerve gives no branch to the artery. The median nerve is seen giving a branch to the interosseous artery in the upper part of the forearm and also supplying a branch to the radial artery at the wrist. The ulnar artery receives a branch from the ulnar nerve (found in fifteen cases).

Fig. 9. The ulnar nerve supplying two branches to the ulnar artery in the forearm (found in two cases).

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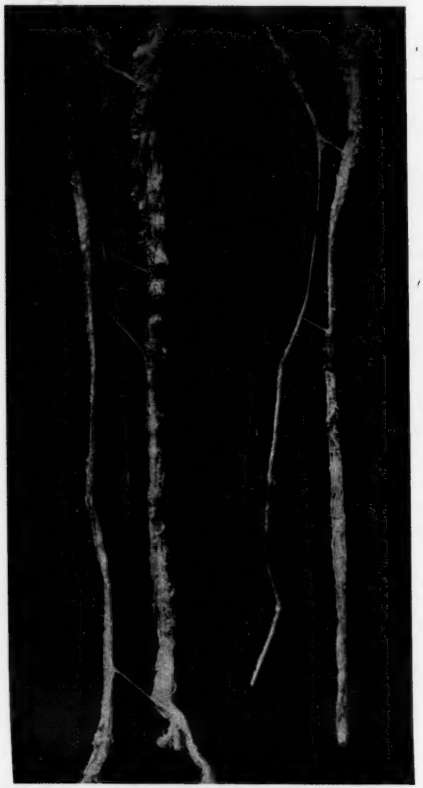

Fig. 10.

Fig. 11.

Fig. 10. Dissection of the posterior tibial artery (left-hand side) showing several branches of the tibial nerve. On the right side, the anterior tibial artery is seen receiving branches from the deep peroneal nerve.

Fig. 11. Microphotograph of a small branch of the tibial nerve joining the posterior tibial artery. Stained with gold chloride. Note the large medullated nerve fibres, also the small fibres presumably of sympathetic origin. This specimen was removed from the amputated leg of a man suffering from thrombo-angiitis. The lumbar sympathetic ganglia had been removed

Fig. 10.

Stained with gold chloride. Note the large medullated nerve fibres, also the small fibres presumably of sympathetic origin. This specimen was removed from the amputated leg of a man suffering from thrombo-angiitis. The lumbar sympathetic ganglia had been removed

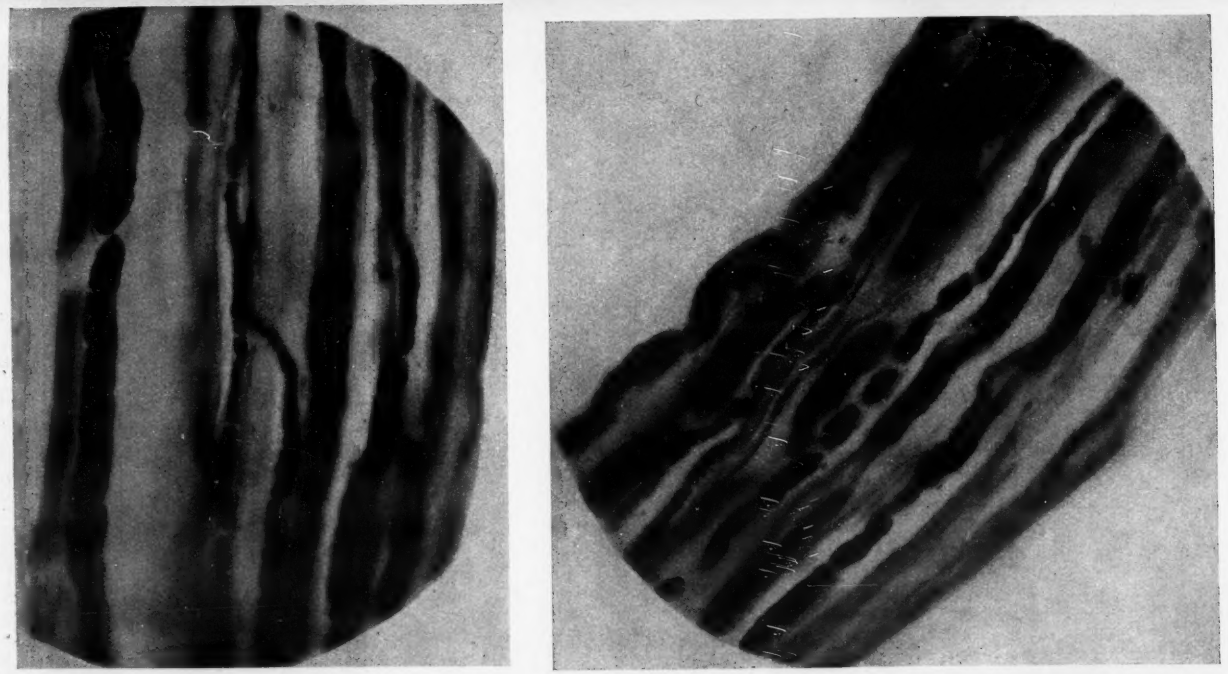

Fig. 13. Two microphotographs of branches of the tibial nerve joining the posterior tibial artery. Same case as in fig. 2. Stained with gold chloride. Note the degenerated small nerve fibres probably due to sympathetic ganglionectomy. 10 days before amputation. The medullated nerve fibres show changes suggesting degeneration also.

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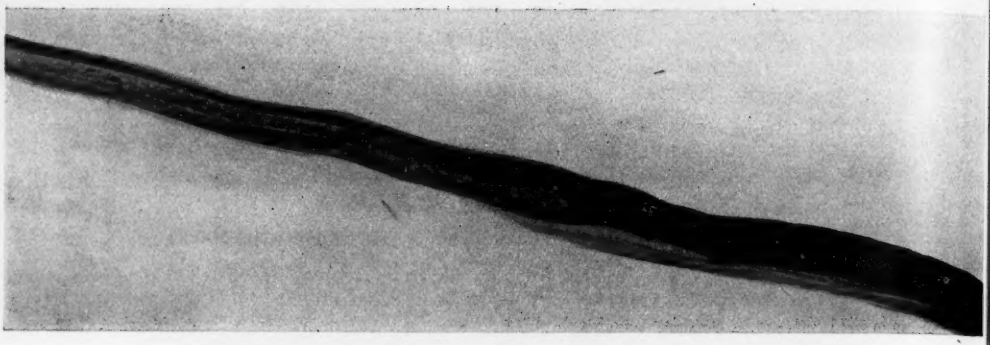

Fig. 12. Unstained medullated nerve fibre isolated from the first small branch femoral nerve to femoral artery. From specimen shown in fig. 4.

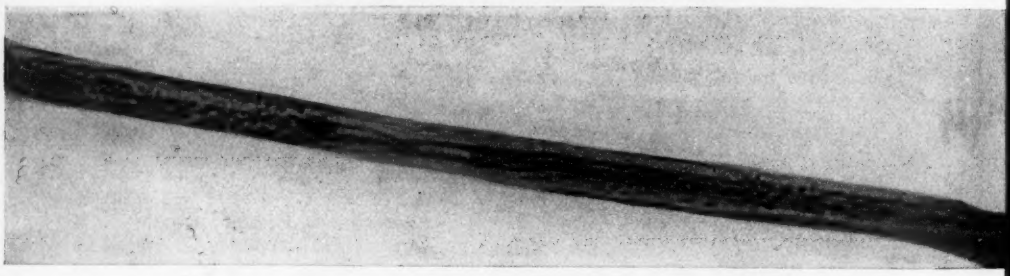

Fig. 14. Unstained isolated nerve fibre of medullated type from second branch of femoral nerve as seen in fig 4.

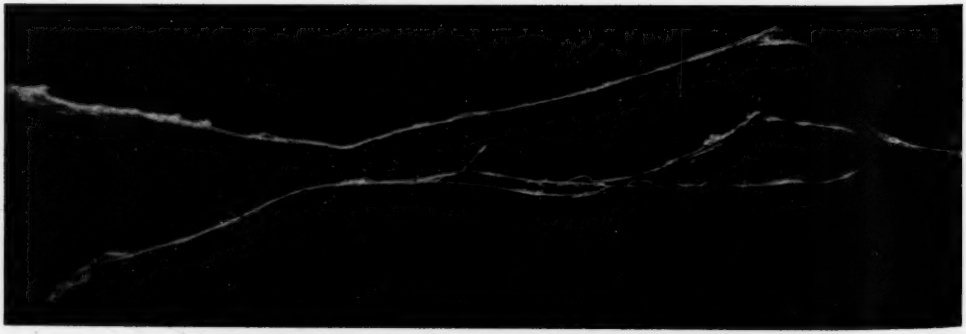

Fig. 15. Branch of the radial nerve passing to the cephalic vein in the forearm.

The anterior tibial artery receives one or more branches of the accompanying deep peroneal nerve.

The nerve supply to the large peripheral veins was not examined in detail, but one large branch of the superficial radial nerve was found passing on to the cephalic vein in the forearm (see fig. 15).

The function of the small nerves above described is not thoroughly understood, but there is evidence suggesting that injury to these nerves or section of their fibres in the parent trunk produces trophic changes in the limbs.

In a case of attempted suicide, the ulnar nerve was cut in the lower third of the forearm, the ulnar fingers were found to be warmer than the thumb and index finger for four days, then the temperature in those fingers fell below that of the remainder of the hand, and the usual trophic changes appeared. This phenomenon has been observed by other writers, notably Sir Thomas Lewis (3). The explanation, that the irritation of the intact cut end of the nerve produces a vaso-dilatation, to be followed later by a vaso-constriction when nerve degeneration has occurred, appears to be the correct one. The observation that after peripheral nerves have been cut or torn (rupture of the brachial plexus) the first evidence of return of function is seen in the improved trophic conditions of the limb, suggests that the regeneration of the vascular branches of the nerves precedes that of the skin supply (4).

The extensive supply of nerve fibres from peripheral nerves to the limb arteries found in this series confirms the work of Woollard (5).

Recent examination of the arterial nerve supply has shown that the periarterial sympathetic plexus passes only a short distance down the arterial trunk in the limbs.

I am indebted to Prof. F. Wood-Jones for advice in the preparation of this paper.

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THE MORPHOLOGY OF THE STERNUM AND ITS RELATION TO THE RIBS

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In a paper previously published in this *Journal*, we described cases of cervical ribs and rudimentary first thoracic ribs considered from the clinical and etiological standpoints and noted the varying relations of these abnormal ribs to the sternum.

In considering the causes of these variations we were driven to the conclusion that one of the first steps towards the solution of the problem was to compute, as far as possible, the factors which are concerned in determining the normal relationships of the rib cartilages and the sternum, one to the other.

Are the articulations of the ribs with the sternum primary or secondary? Is the human sternum developed by fusion of the upturned ends of the rib cartilages or do the ribs take no part in its formation? How is the sternum developed in cases where the ventral ends of the ribs are defective and fail to reach the sternum? It is evident that these and other subsidiary questions can only be satisfactorily answered by a general survey of the origin of the sternum from its earliest appearance in the more primitive types of vertebrates, both living and extinct, and a comparison of these with higher mammalian types including that of Man.

The following account of our work is an attempt to co-ordinate and harmonise the many conflicting points at issue, and to define the names which are applied to the various component elements of the sternal apparatus.

THE STERNAL APPARATUS

It is desirable before entering into the question of the development of the sternum to preface our remarks by a definition of the names applied to the separate elements which are concerned in the formation of the sternal structures and the closely associated ventral segment of the shoulder girdle.

These may be considered under the following headings:

(1) The sterno-coracoid membrane and the mesodermal sternal bars.

(2) The coracoidal elements of the pectoral girdle:
- (*a*) Procoracoid (precoracoid).
- (*b*) Coracoid (postcoracoid).
- (*c*) Epicoracoid.

(3) The clavicles and interclavicle.

(4) The episternal process, or omosternum.

(5) The true sternum, which in some Reptiles and Mammals shows a subdivision into:

(*a*) Presternum.
(*b*) Mesosternum.
(*c*) Metasternum.

(6) Parasternalia, or abdominal ribs.

The adjectives "prozonal," "zonal," and "metazonal" are very generally employed by morphologists to denote the relation that the various parts of the sternal apparatus bear to the shoulder girdle. It must be borne in mind, however, that an element which primarily belongs to one of these regions, e.g. the "zonal," may extend forwards or backwards and, becoming segmented off from the original stem, may form an apparently independent element in one of the adjacent zones. In these cases, the true nature of such an element can only be determined by a careful study of successive stages in its ontogeny. Moreover, a particular part may be compound in origin, the primary structure having been invaded, or covered by secondary elements, e.g. the sternum by the ventral ends of the ribs or the interclavicle. The primary elements may be modified in various directions, according to the requirements of the animal. Thus a particular element may be further developed as is the case in the formation of a median keel and the cornua of the true sternum in birds. On the other hand, a part may undergo reduction, e.g. the episternal elements in most of the higher Mammals. Variations also occur in the extent to which the different parts of the sternal apparatus become ossified, and in the presence or absence of serial segmentation of the true sternum in mammals.

The sterno-coracoid membrane is a name introduced by Engler to designate the fibrous or fibro-elastic membrane which unites the ventral ends of the coracoids across the median plane. It is well seen in *Proteus anguineus*, in which it remains membranous throughout life. In the embryo, it is represented by a stratum of condensed mesoderm which is developed in the pre-cardial region between the two halves of the developing pectoral girdle. In many of the Amphibia, Reptiles and Birds, and in some Mammals, this membrane is invaded by a medial extension of the inner cartilaginous borders of the coracoids. In some animals the clavicles and interclavicular structures are developed in close relation with the anterior border of the sterno-coracoid membrane.

The mesodermal sternal bars or sternal plates are the forerunners of the sternum. They are bilateral longitudinal thickenings of the mesoderm which are developed in close association with the inner ends of the coracoids and the ventral ends of the ribs. They are also intimately related to the origins of the pectoral muscles which spring from the ventro-lateral border of each sternal bar.

The *procoracoid* is the anterior part of the ventral segment of the pectoral arch.

The *coracoid* is the posterior part of the ventral segment of the pectoral arch.

The *epicoracoid* is the medial element of the shoulder girdle.

The *procoracoid* (fig. 1), also called the "precoracoid," arises in Urodeles from the anterior border of the coraco-scapular cartilage, in the region of the glenoid cavity. The procoracoid projects forwards, or forwards and medially, in such a way that a notch, the *incisura coracoidea*, is formed between it and the inner part of the coracoid. In Urodeles, the supracoracoid foramen, which transmits the nerve of the same name, lies between this notch and the glenoid cavity. In higher types of vertebrates the procoracoid may become ossified and may take part in the formation of the clavicle. In tailed amphibians the *coracoid* and *epicoracoid* (fig. 1) are not differentiated, the part, which corresponds to the epicoracoid in the Anura, being represented merely by the medial part of the main coracoid cartilage. In Salamandra the medial borders of the two

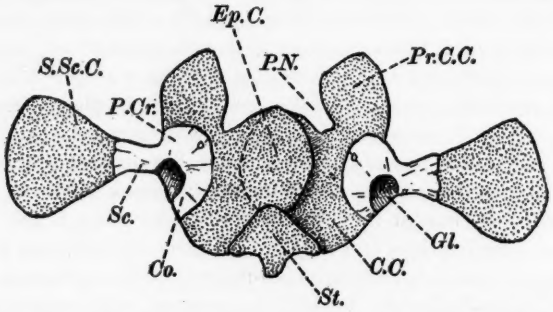


Fig. 1. Shoulder girdle and sternal apparatus of *Salamandra atra* viewed from ventral aspect. The ossification of the scapula coracoid and procoracoid around the glenoid cavity is continuous. The precoracoid notch and foramen indicate the line of separation between procoracoid and coracoid. The overlapping inner borders of the continuous cartilage represent the epicoracoids. The sternum is wholly cartilaginous.

coracoid cartilages overlap each other; while in *Proteus anguinea* and *amphiuma* they are separated by a wide interval which is occupied by the sterno-coracoid membrane. In adult Anura, the part adjoining the glenoid cavity frequently becomes ossified to form a separate element, the coracoid, while the remaining unossified part forms the epicoracoid. The epicoracoids may fuse in the median plane (Firmisterna) or overlap each other (Arcifera). They may be prolonged forwards and thus give rise to an episternal process or backwards and thus exhibit right and left lateral lobes, as in *Xenopus* and *Pipa Americana*, or their ends may become separated off from the main part and exist as apparently independent elements, e.g. the sternal leaflets of *Bombinator* which, as has been shown by de Villiers, arise on each side as a separate chondrification in a blastema which is common to the leaflet and the epicoracoid.

The *clavicles* and *interclavicle* are dermal bones which, from the phylogenetic standpoint, are very ancient structures, and appear in well-developed

forms in certain extinct Reptiles in the Permian strata of South Africa and Madagascar (fig. 2). The dermal or membranous clavicle is frequently so blended with the cartilaginous precoracoid as to form with it a single bone, examples of which are the clavicle of many Mammalia and that of the human skeleton. The interclavicle, which is highly developed in certain Reptiles such as the Crocodilia, is vestigial or absent in eutherian Mammals. In alligators, the interclavicle extends a considerable distance caudally on the superficial surface of the presternum, and, as has been shown by J. A. Kalin, is developed as a bilateral structure independently of the sternum.

The *episternal process* (omosternum) is well developed in many Amphibia in which it is formed as a forward prolongation of the epicoracoids. It is cartilaginous in origin and may remain in continuity with the epicoracoids or be segmented off and form an apparently separate element. In *Rana* the episternal

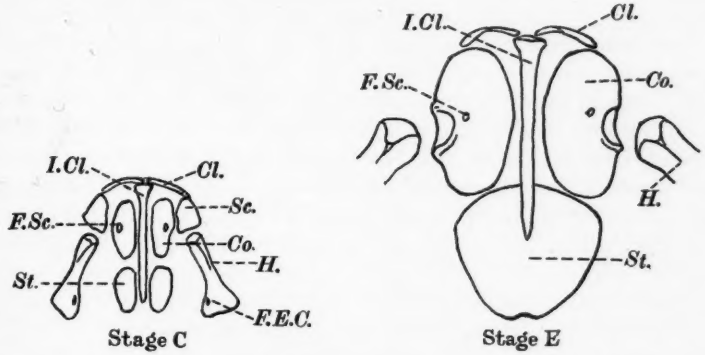


Fig. 2. Pectoral girdle and sternum of *Tangasaurus Menelli*. Ventral aspect. Stage C shows the sternal plates situated behind the coracoids, on each side of the posterior end of the interclavicle. Stage E, from a specimen of an older animal, shows the two halves united in a single sternal plate. (After J. Piveteau.)

process gives origin to the coraco-radialis muscle and to the episternal part of the deltoid. It must not be confused with the interclavicle, which is membranous in origin, primarily superficial to the muscles, and in derivation quite independent of the epicoracoid cartilages. Unfortunately the term "episternum" is frequently applied both to this element and to the interclavicle which is a dermal structure. The latter is obviously not homologous with the episternal process or "omosternum" of Parker which is primarily derived from cartilaginous elements of the pectoral girdle.

The true *sternum* is the most caudal part of the sternal apparatus, and is often described as being "post-zonal" in position. It is believed by some authors to be represented in certain Fishes by the "post-omosternum," or the posterior part of the interclavicular cartilage which is present in *Notidanus* and *Ceratodus*. In Urodeles, in which a sternum is present, it is wholly cartilaginous

(fig. 1), and its edges are grooved by the inner borders of the coracoid cartilages. In certain Anura the sternum is partly ossified, partly cartilaginous, e.g. *Rana*. In other Anura it is represented by two diverging horns which are joined anteriorly and form a Λ-shaped structure, as is the case in *Bombinator igneus*. These horns probably represent the commencement of the sternal bars which are so highly developed in certain Reptilia, whereas the apex of the Λ, where the two bars become fused medially, represents the principal element of the *presternum*. In *Chirotes canaliculates*, as has been pointed out by Hanson, the proximal ends of the two bars of the Λ are joined medially and form a *mesosternum*, whereas the distal ends of the two bars diverge and provide a bifid *metasternum*.

The *parasternalia*, or abdominal ribs, are slender curved bones which usually lie superficial to the rectus muscles of the abdominal wall and behind the sternum. They are primarily membranous in origin and are regarded by some morphologists as not being homologous with the true ribs. They present a Λ-shaped form; there are frequently two or more in each segment. It is generally supposed that they correspond to the posterior segments of the plastron of the chelonian Reptiles. In certain extinct Reptiles they may co-exist with the oval bilateral plates which are situated posterior to the coracoids and which represent the true sternum, e.g. *Tangasaurus* (fig. 2) (J. Piveteau). These bilateral plates are fused to such an extent in older specimens of the same species as to form a single median plate which does not appear to have been connected at any stage with either the true ribs or with the slender parasternalia. It may be noted, however, that these abdominal ribs closely resemble in form the Λ-shaped sternal cornua of *Bombinator* and of living Reptiles. The abdominal ribs are, however, much more slender and more numerous.

The sternal cornua are a single pair of diverging processes and are never multiple. They are, moreover, cartilaginous in origin, not membranous, and are directly continuous anteriorly with the true sternum. The cartilaginous bands which are present in the myocommata of the rectus abdominis muscles of *Necturus* are generally regarded as vestiges of true ribs. They are frequently described as "abdominal ribs," but some authors prefer the term "sternal ribs," this name implying a supposed primary connection with the sternum.

If these points with regard to nomenclature are carefully considered, it will be realised that the controversy, which has arisen with regard to the origin of the sternum as a whole, becomes grouped into several distinct points of issue and becomes limited to the mode of development of particular parts of the sternal apparatus. More especially the extent to which the component parts of the sternum are laid down in that part of the ventral wall of the thorax or abdomen which gives origin to the sterno-coracoid membrane, and which more posteriorly is represented by the linea alba. Further the extent to which the invading elements of the shoulder girdle and ventral ends of the ribs participate in the formation of the sternum.

The ontogeny of its individual elements must be studied in different types

of animals in the light of comparative anatomy and with due regard to functional requirements, in order that the more general question of the phylogeny of the sternum can be profitably discussed, and more particularly, the problem whether or not the true sternum of the Amniotes is to be considered as homologous with that of the Anamnia.

Morphological relation of the ribs to the sternum

In order to understand the irregular, often bizarre, relations of the rib cartilages to the sternum in cases of variation in number of the ribs, e.g. cases of cervical ribs, rudimentary first thoracic ribs, or fusion of two or more ribs, it is desirable to consider first the normal relations of the ventral ends of the ribs to the sternum both from the phylogenetic and ontogenetic standpoints. A consideration of the varying relations of the rib cartilages to the sternum in abnormal cases is also instructive as affording an illustration of the occurrence of secondary adaptive changes in the later developmental phases of the embryo which compensate, to some extent, for an earlier primary disturbance in ontogeny of the normal processes of segmentation.

The origin and comparative anatomy of the sternum has been studied by many workers, of whom special mention may be made of Rathke, Wiedersheim, the Parkers, Paterson, and Hanson; and its ontogeny chiefly by Goette, Ruge, Paterson, Charlotte Müller, Kravetz, Hanson, Whitehead and Waddell, Mary Juhn (Lacerta), de Villiers (Anura), Engler (Urodeles) and Kalin (Crocodilia). We have thought it necessary, however, before describing the development of the sternum, to insert the following brief account of its comparative anatomy, with the object of recalling and commenting on certain main facts concerning its morphology and its relation to the ribs, rather than an attempt to add anything new. The account is based partly on our personal observations and partly on the published works of the above-mentioned authors. It is intended to indicate what, in the opinion of the writers, may be regarded as some of the more essential facts bearing upon the phylogenetic origin of the sternum and of the developmental relationship of the ribs to this bone.

For a more detailed account of the phylogeny of the sternum, the reader should consult the special papers concerning the sternum in the Anura, Urodela and Reptilia mentioned above and a more general survey in an article by F. B. Hanson in the *Amer. J. Anat.* vol. XXVI, 1919. In his paper, Hanson discusses evidence for the origin of the sternum as a structure primarily arising in connection with the shoulder girdle and traceable as such through all classes of the Vertebrata, as opposed to the dual origin which has been assigned to it by some morphologists, namely "coracoidal" in the Anamniota, "costal" in the Amniota. The latter authors, accepting Ruge's view of its origin from the ribs and being unable to reconcile this hypothesis with the evidence of comparative anatomy, conceived the idea that the sternum of the Amniota, being as they supposed purely costal in origin, differed morphologically from the

sternum of the Anamniota which they recognised as either undoubtedly "coracoidal" in origin, or as a structure formed independently in the "linea alba." The ontogenetic development of the sternum in those animals such as the pig, in which the coracoid and clavicle are either absent or rudimentary and do not reach the sternum, has hitherto offered an apparently serious obstacle to the full acceptance of the view that the sternum has, in these animals and in Man, originated, as in the Ichthyopsida, from two mesodermal bars and that the participation of the ribs in its formation is a secondary rather than a primary factor. In our attempt to solve this difficult problem we have, therefore, given considerable attention to the early stages in the ontogeny of the sternum in Man and in certain Mammals, and also in representative types of Amphibia, Reptiles and Birds. Moreover, we have endeavoured to deal with the developmental side of the question in a more detailed manner than appears to be necessary from the phylogenetic standpoint, since most of the essential facts bearing on the comparative anatomy of the sternum are already known. The episternal structures in the Ichthyopsida have been definitely shown to arise in close relation with the shoulder girdle; while the true sternum including the metasternum originates as processes of the episternal structures and extends backwards into the thoracic and abdominal walls.

We find that while there is a general agreement in the essential principles of the development of the sternum throughout the different classes of vertebrate animals, the details of its ontogeny differ almost as widely as the details of its structure in adult animals. This is more especially the case with regard to the extent to which the episternal and coracoidal elements are developed, and the degree to which the ribs may be considered as entering into the composition of the true sternum. We may state at once that we are confident that the evidence of comparative embryology is in complete agreement with that of comparative anatomy.

A study of its ontogeny shows that the sternum arises in the higher Vertebrates by the formation of two bilateral bars or plates of condensed mesoderm. These are at first widely separated from each other and lie in the region of the axilla, posterior to the ventral segments of the shoulder girdle and in close relation with the developing pectoral muscles which primarily arise in association with the mesodermal sternal bars (fig. 3). In those animals in which ribs are present, the ventral ends of the ribs grow towards and join the mesodermal sternal bars. The sternal bars afterwards move inwards into the precardial region where they unite anteriorly with a mass of condensed mesoderm. This is median in position and occupies the interclavicular area. Subsequently, the mesodermal bars unite with each other from before backwards in the median plane and form a median plate which is prolonged caudalwards as the xiphisternum, or sternal cornua. The anterior median condensation of the mesoderm is primarily bilateral and represents the epicoracoids and episternal process of lower types of Vertebrates, such as the Amphibia. There is also a median condensation of the mesoderm which lies more ventrally. This probably represents

the interclavicle of the Monotremes, Reptiles and extinct Amphibia. It must be borne in mind, however, that the interclavicle appears at a somewhat later stage in ontogeny than that in which the medial condensation first appears in the embryos of those animals in which it is a fully developed structure in the adult, e.g. in the Crocodilia. The details in the mode of chondrification and ossification of the sternal bars and sternum differ markedly in different types of animals and will be dealt with separately after the general description of its comparative anatomy.

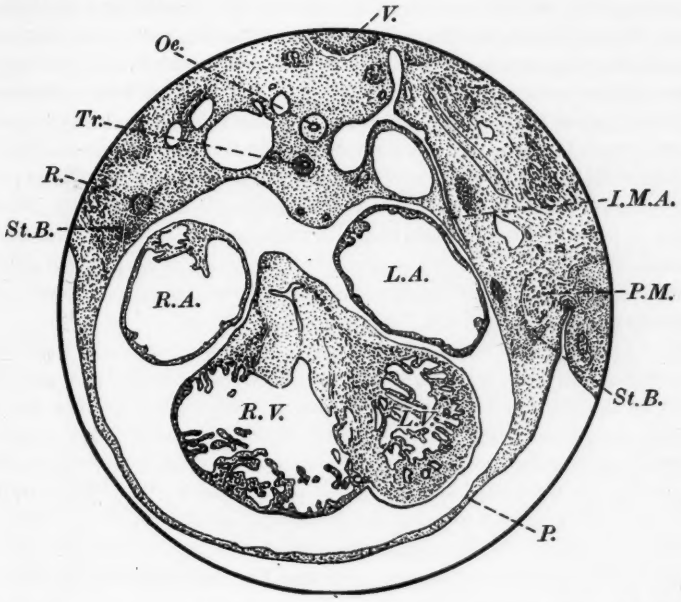


Fig. 3. Transverse section through a cat embryo (12 mm.), showing the position of the mesodermal sternal bars relative to the axilla, pectoral muscles, ribs and pericardium.

A short resumé of the comparative anatomy and phylogeny of the sternum

Before describing our observations on the ontogenetic development of the sternum, it is desirable to give a brief summary of the evidence which is available from the examination of palaeontological material and from the study of the sternal apparatus, shoulder girdle and ribs of representative types of living animals.

(1) *Pisces.* Until recently, the sternum was stated to be absent in most Fishes. In (*Hexanchus*) the Perlon shark (*Notidanus indicus*), *Heptanchus*, and *Acanthias*, however, the ventral or inner ends of the coracoids are united by a median cartilage. This intervening cartilage is believed to represent the sternum. In *Acanthias vulgaris* pre- and postomosterna are also recognisable. Moreover,

in a Teleost (*Gobius niger*), and in the Dipnoi (*Polypterus*), (*Ceratodus* and *Lepidosiren*) epicoracoidal elements which are said to represent the epicoracoids and the pre- and postomosterna of Amphibia are found in the same situation as in the Elasmobranchs. In *Raja*, the two coracoids are joined across the median plane in such a way as to form the ventral segment of the complete pectoral girdle, the dorsal segment of which is constituted by a cartilaginous dorsal plate which bridges across the space between the scapular cartilages and is attached in the median plane to the dorsal spines. It is obvious that this complete girdle, which resembles the pelvic girdle, serves as a firm basis of support for the large lateral fins of this fish and that it, at the same time, serves by its ventral segment to protect the heart and pericardium and give origin to the powerful muscles which move the fins, in much the same way as the sternum in higher types of Vertebrates covers the heart and gives origin to the pectoral and other muscles. Thus, as was described by Paterson, "in certain fishes, the elements of the shoulder girdle meet in the ventral wall of the body and provide a shield for the protection of the heart and branchial chamber. In *Acanthias vulgaris*, a continuous cartilaginous bar extends across the middle line between the confluent lateral halves of the shoulder girdle, and in *Protopterus annectens* there is also a median element which is separate from the coracoids and receives the inner ends of the clavicles."

(2) *Amphibia.* The sternal apparatus in Amphibia varies considerably in different forms, being of a simple type in the Urodela but highly complex in certain of the Anura. Important transitional forms exist between the types which are chiefly aquatic, and those which live chiefly on land. The simple type of sternal apparatus met with in the tailed Amphibia, and the transitional stages between these and the sternal structures present in the more highly evolved forms such as *Rana* and *Bombinator*, furnish valuable evidence with regard to the origin of the sternum and illustrate very clearly the close dependence of structure on function, and also the lines along which the whole class of Amphibia has evolved.

It will be convenient to commence the description of the sternum of the Amphibia, with a highly evolved type of sternal apparatus, such as is present in the common frog. Briefly this consists of:

(i) An anterior part, or episternal process. This is a median element, the base of which is bony and articulates behind with the epicoracoids. Anteriorly, it broadens out into a flattened disc of cartilage.

(ii) An intermediate part formed by the fusion in the median plane of the right and left epicoracoid cartilages. This articulates on each side with the inner ends of the coracoids which are of large size and ossified.

(iii) A posterior part, the true sternum. This is median in position, its basal part, which is bony, articulates with the fused epicoracoid cartilages; the caudal extremity ends in an expanded disc of cartilage.

Two views are held regarding the ossification of the procoracoid in *Rana*, namely that of W. K. Parker and Goette who hold that there is a perichondral

and endochondral ossification of the procoracoid, similar to that of the coracoid; and that of Gegenbaur who believed that the ossification is wholly dermal in origin, a thin layer of connective tissue separating the bone from the underlying cartilage. If Gegenbaur's hypothesis is correct the membrane bone must have sunk beneath the muscles, or the muscles have extended over the bone and thus separated it from the skin. That the sinking of a primarily dermal bone beneath the surface is a possibility is illustrated, however, by such bones as the maxilla and mandible which are covered by the muscles of the face. Our specimens support Gegenbaur's contention that a thin layer of fibrous tissue lies between the covering bone and the procoracoid cartilage (fig. 4), but we do not consider that this bone is entirely dermal in origin, since the ossification also involves the procoracoid cartilage situated deeply to the membrane bone.

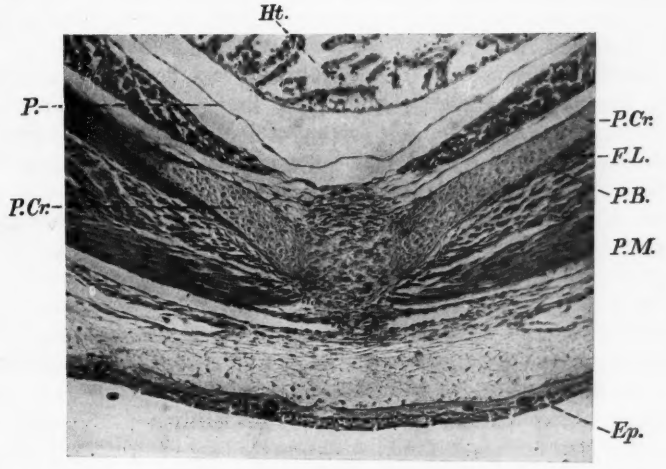


Fig. 4. Transverse section through a tadpole of *Rana temporaria*, showing the formation of ecto-steal bone on the surface of the procoracoid cartilage. A thin stratum of fibrous tissue intervenes between the "covering" bone and the underlying cartilage. The mesodermal sternal bars have united in the median plane and have been converted into a median bar of procartilage.

In Urodela, a more primitive type of shoulder girdle and sternal apparatus is met with than that in the Anura. Nevertheless, in spite of this simplicity of structure, differences exist in the degree of development of the coracoids and sternum which depend, as has been shown by Engler, on the habits of the particular species. Thus in purely aquatic types of Amphibia, in which the limbs are used merely as paddles and are not required to support the weight of the body on land, the coracoid and scapula are mostly cartilaginous structures, a small part only, near the glenoid cavity, being bony (fig. 1). If a sternum be present, it is completely cartilaginous.

This condition is well represented in *Salamander atra*, which, although it undergoes a complete metamorphosis with the loss of its gills, yet spends the greater part of its life in the water, only coming on land for short periods at a time. The coracoids overlap each other across the median plane (arcifery), as in certain of the Anura, and there is a well-marked notch opposite the supra-coracoid foramen, separating a procoracoid part of the cartilage from the main posterior part which articulates with the sternum and represents the coracoid of certain of the Anura.

In *Proteus anguineus*, the coracoids do not reach the median plane but are connected together by a fibrous membrane which bridges across the intervening space. This is the *membrana sterno-coracoidea* (Engler). A slender procoracoid process is prolonged forwards from the anterior border of the main portion of the coracoid with which it is continuous. The base of the procoracoid process is, however, separated from the coracoid by a deep notch similar to that found in *Salamandra atra*.

In *Necturus* (*Menobranchus*), a condition is present which is intermediate between that of *Salamandra atra* and that of *Proteus*. The cartilaginous coracoids meet in the median plane or overlap each other to a slight extent only. The procoracoids jut forwards from the anterior border of the main part of each coracoid from which they are separated by a wide rectangular notch (incisura coracoidea). Pairs of slender cartilaginous bars (parasternal cartilages or "abdominal ribs") extend laterally into four or five of the *inscriptiones tendineae* from median basal cartilages situated in the *linea alba*. In the coracoidal region the basal cartilages tend so to fuse with one another as to form a median longitudinal bar. This was considered by Wiedersheim to represent the mode of origin of the true sternum, while he believed the lateral extensions into the myocommata to be vestigial ribs.

In tracing the phylogeny of the sternum, the position in the ancestral tree of *Menobranchus*, *Proteus*, *Amphiuma* and other apparently lowly organised forms of Amphibia must be very carefully considered. Three alternative views with regard to their origin may be discussed:

(*a*) Are they to be regarded as primitive, in the sense that they have reverted from a more highly organised condition to a simpler ancestral form?

(*b*) Is their low organisation to be explained by assuming that their degenerate form is simply due to loss of function and that the degenerative changes have been uninfluenced by heredity?

(*c*) Are they "primitive" in the sense that the type has never evolved beyond the state in which it exists at the present day?

If the first proposition is accepted, it must be borne in mind that there is a likelihood of the reversion not being along lines exactly similar to those of the evolution. That is to say, in descending the steps by which the evolutionary tree has been built up, there is a probability of deviation from the original path, and certainly there is no evidence that the descent will correspond inversely to the ascent.

If the second hypothesis is adopted, the present structural condition of these animals cannot be relied upon as a safe indication of the condition in early ancestral forms.

The third assumption that these animals are truly primitive and not degenerate is negatived by many structural details of their organisation, and more especially the condition of the limbs and sense organs.

We consider, therefore, that, although the cartilaginous bars which are present in the *linea alba* and *lineae tendineae* of *Menobranchus* are suggestive of the view that in these animals the origin of the sternum resulted from the fusion of the ventral ends of ribs, too much reliance should not be placed on this circumstance. Consideration must also be given to the formation of the sternum in other types of animals such as *Rana* in which the ribs do not extend into the sternal region in living species, and in which there is no certain evidence that they have reached the sternum in extinct animals of the same class. The evidence of palaeontology, more especially that of certain extinct Reptiles such as *Youngina capensis* (Broom) and *Tangasaurus* (Piveteau), is strongly in favour of the origin of the sternum as two bilateral plates of bone, situated behind the coracoids and formed quite independently of the ribs.

Between the highly evolved sternal apparatus of *Rana esculenta* and the simple arrangement met with in *Salamandra atra* is a most instructive intermediate form, that of *Pipa Americana* Laur. In this animal, the inner ends of the procoracoids have been entirely replaced by the clavicles, only a small part of the procoracoid cartilage remaining externally in relation with the glenoid cavity. The epicoracoid cartilages have been prolonged forwards in front of the inner ends of the clavicles, in the situation of the episternum of *Rana*. This forward prolongation of the epicoracoids, however, is not separated to form a distinct element, as in *Rana*, but remains in continuity with the chief part of the cartilage which corresponds to the intermediate part of the sternal apparatus in *Rana esculenta*. The sternal cartilage is intermediate in type and articulates by its antero-lateral borders with the epicoracoid cartilage on each side. The coracoid or "obturator foramen," between the procoracoid, coracoid and epicoracoid cartilage in this and other species, may be regarded as an unchondrified and unossified area of the membrana sterno-coracoidea (Engler). The bilateral origin of the sternal anlage will be dealt with later, when the ontogeny of the sternum in the Amphibia is described.

Other important modifications of the sternal apparatus in the Amphibia, produced by the two processes of development or suppression of particular parts according to the functional requirements of the animal, are admirably illustrated in *Xenopus*, *Bufo*, *Rana natatrix*, *Microhyla*, *Hemisus sudanense*, and *Dendrobates tinctorius*.

The epicoracoid cartilage of *Dendrobates tinctorius* lies between the fully ossified clavicles and coracoids, and closely resembles the interclavicular cartilage of *Ceratodus* to which it may be regarded, not only as an analogous, but also as an homologous structure.

In both *Dendrobates* and *Ceratodus* the single median cartilage, present in the adult animal, has obviously been formed by the fusion in the ventral median plane of the right and left halves of the pectoral girdle and may thus be described as "coracoidal" in origin, using the term coracoidal in a wide sense as implying both the primary coracoid cartilage and its derivatives, the procoracoid and epicoracoid cartilages.

The high degree of development of the sternal apparatus in the adult frog has already been alluded to and it appears obvious that it is evolved as a median support for the attachment of the pectoral and abdominal muscles and affords protection for the heart and lungs. The whole sternal apparatus is thus comparable with the structures in the mid-ventral line of the pelvic region, namely the symphysis pubis, epipubis and hypoischinum. The atrophy of the sternum which has accompanied the degeneration of the forelimbs in such types

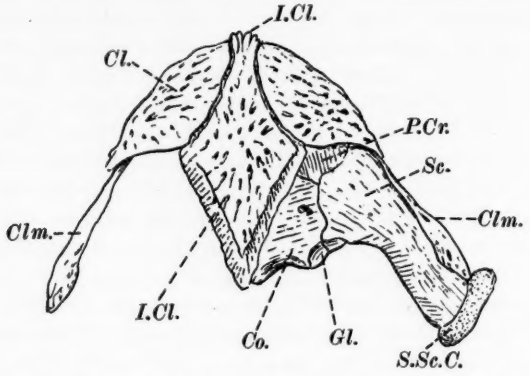


Fig. 5. Shoulder girdle and sternal apparatus of *Archegosaurus*. (After O. Jäkel.)

as *Proteus* and *Amphiuma* is additional evidence of the close association of the sternum and episternum with the shoulder girdle.

In a general way, the degree of development of the sternal apparatus in the Amphibia corresponds to the degree of development of the shoulder girdle and the pectoral muscles. The basis of support which the sternal structures give to the shoulder girdle is obviously more yielding in nature in the Arcifera and more stable in the Firmisterna.

The interclavicle and the "membranous" or "dermal" clavicle appear to be completely absent in living forms of Amphibia. Both of these bones were present, however, in certain extinct species belonging to the class Stegocephala. The interclavicle occurred as a flat diamond-shaped plate (fig. 5) (*Archegosaurus*), or a fan-shaped bone (*Hylonomus*). It lay between the clavicles and the pre- and postcoracoids, and in some species (*Cacops* and *Discosaurus*) was prolonged backwards as a median bar between the coracoids as in many existing and extinct reptiles. In *Hylonomus* (Credner) it was situated on the ventral

aspect of a calcified plate of cartilage which most probably corresponds to the fused epicoracoid cartilages.

The interclavicle of the Labyrinthodonts (Stegocephala) appears to be homologous with the anterior median plate (entoplastron) of the ventral shield of the Chelonia, whereas the membranous clavicles are most probably homologous with the epiplastra (Rathke, W. Kitchen Parker). No relics of a "true" or postomosternum appear to have been discovered in extinct Amphibia, but it is probable that, if it existed, it was entirely cartilaginous in nature and has, for this reason, not been preserved. The interclavicle is primarily a dermal bone and should not be confused with the primarily cartilaginous episternal process of the Anura which is "coracoidal" in origin. There is no definite evidence of ribs articulating with sternal structures in extinct classes of Amphibia.

(3) *Reptilia.* According to Broom, the oldest known ossified sternum has been found in a primitive Permian reptile, *Youngina.* It consists of two bilateral plates lying behind the coracoids and was probably formed as the result of separate ossifications involving a flat plate of cartilage. In the Dicynodont *Lystrosaurus*, the two plates are so fused as to form a single large quadrangular plaque which supports the coracoids in front and probably received three pairs of ribs which were attached to its postero-lateral borders. Broom considers that behind this plaque there was a median cartilaginous extension which represented the xiphisternum. In the Theriodont *Lycaenops*, the sternum is more elongated, and it is probable that in this animal four pairs of ribs reached the sternum.

A massive T-shaped interclavicle, supporting membranous or dermal clavicles, is a conspicuous feature of the sternal apparatus in *Champsosaurus*, a Diapsid Reptile. The interclavicle and clavicles are also highly developed in *Conodectes*, the interclavicle articulating laterally with the coracoids between which it lies (Broom).

In the therocephalian *Pristerognathus* and *Dicynodon*, the interclavicle is an elongated flattened plate, shaped like a spatula. The clavicles overlap the anterior end of the interclavicle superficially; this end of the interclavicle is not characterised by the pronounced cross-bar which is so conspicuous in *Champsosaurus*. The posterior end of the interclavicle is prolonged backwards on the ventral aspect of the coracoids and overlies the anterior border of the true sternum superficially. The clavicles in *Dicynodon* articulate laterally with a definite acromion process.

The occurrence of two ossified sternal plates, lying posterior to the coracoids in young specimens of *Tangasaurus* (fig. 2) and *Hovasaurus* and their union into a single median plate in older specimens, as described by J. Piveteau, has already been alluded to under the heading "Parasternalia," (p. 512). The sternal plates appear to have been formed quite independently of the parasternalia.

Among living types of the Reptilia, the snakes, which have no anterior limbs or shoulder girdle, are not provided with a sternum. In Lacertilia,

epicoracoid cartilages, interclavicle and sternum are all present. In *Iguana*, the interclavicle is definitely T-shaped and articulates anteriorly with well-developed clavicles. The sternum is prolonged backwards and has two diverging sternal cornua. Four pairs of ribs are attached to the sternum proper, the succeeding ribs being attached to the outer edges of the sternal cornua. In *Gecko*, the cartilaginous epicoracoids overlap each other in the median plane, as is the case in the arciferous Amphibia. They are covered superficially by a diamond-shaped interclavicle which articulates in front with dermal clavicles. The posterior end of the interclavicle is prolonged backwards on the ventral

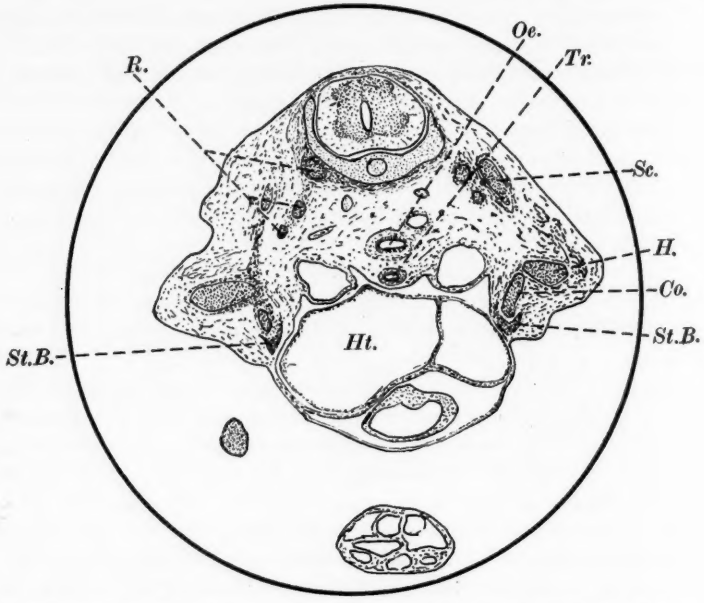


Fig. 6. Transverse section through an embryo *Gecko*, showing the relation of the mesodermal sternal bar to the coracoid cartilage and pericardial wall.

aspect of the sternum and two horn-like processes of the epicoracoid cartilages project outwards in front of the lateral angles of the sternum. The sternum is prolonged backwards as two sternal cornua. Three pairs of ribs join the sternum in the adult, and a fourth pair articulate with the sternal cornua. In an embryo *Gecko*, belonging to the King's College collection (fig. 6), the first two pairs of ribs have not reached the mesodermal sternal bars which project forwards a considerable distance beyond the tips of these ribs. There is no indication of there having been an earlier primary connection between these ribs and the sternal bars; consequently the connection of these ribs with the sternum in the adult must be regarded as secondary. In *Sphenodon*, the sternum lies well

forward in the posterior region of the neck. Only two or three pairs of costal cartilages reach its lateral borders. Anteriorly it articulates with and gives support to well-developed coracoids and clavicles. The ventral ends of the posterior thoracic ribs turn forward and articulate with one another and thus form two jointed longitudinal bars which extend backward almost as far as the pelvis.

In Crocodilia, the sternum consists of a simple elongated plate of cartilage which anteriorly lies on the dorsal aspect of the interclavicle and posteriorly is prolonged backwards as two xiphisternal horns. In *C. palustris*, eight pairs of ribs articulate with the sternum and its cornua. Behind these are eight or more pairs of slender membrane bones, the "abdominal ribs," which approach each other in the median plane and present Λ-shaped angles, with the point of the Λ directed towards the sternum.

In *C. vulgaris*, there are ten pairs of ribs which reach the sternum, cranial to these are nine (?) pairs of cervical ribs. The last two pairs of cervical ribs and the eighth and ninth vertebrae which support them are, however, thoracic in character, and although these ribs do not reach the sternum they are properly regarded as rudimentary thoracic ribs rather than as cervical ribs. If this view is taken of the nature of the eighth and ninth pairs of ribs, the number of cervical vertebrae in the crocodile is seven and corresponds with the number generally found in the Mammalia. The Crocodilia, however, differ from the Mammalia in possessing a complete series of seven bicipital cervical ribs. A thoracic rib in the crocodile, with the exception of those of the last two pairs, consists of three parts, a vertebral, an intermediate and a sternal rib. The proximal ends of the vertebral ribs articulate by two heads with the transverse processes of the vertebrae; one head situated ventrally, the "capitulum," articulates with a notch on the side of the transverse process near its base, the other head situated dorsally, the "tuberculum," articulates with the tip of the transverse process. Near the distal end is an imperfectly ossified uncinate process which is directed backwards. The sternal ribs are so expanded that a rib which is more posterior in the series partially overlaps the rib next in front. Their ventral extremities are connected by joints with the posterior end of the sternum and with the outer borders of its xiphisternal cornua.

In the *Chelonia*, no sternum belonging to the endoskeleton is present, but its protective function is vicariously replaced by the "plastron." This is formed by a symmetrical series of paired and unpaired bony plates (epiplastra, entoplastron, hyoplastra, hypoplastra, and xiphiplastra). The carapace, intermediate or marginal plates, and the plastron are all membrane bones. There is, however, a very intimate relation of the carapace (exoskeleton) with the underlying ribs and vertebral column (endoskeleton) and a similar relation of covering bones to the endoskeleton exists on the ventral aspect of the body. No true sternum appears to be present in the endoskeleton. There are, however, a well-developed bony coracoid and a procoracoid, each of which articulates

by means of cartilage with the plastron. This intervening cartilage between the plastron and the coracoids is obviously epicoracoid in nature. The continuous cartilage which joins the procoracoid with the coracoid in lower types has become interrupted by degeneration of the intermediate part, thus leaving a portion in front in relation with the procoracoid (or acromion) and a portion behind in relation with the coracoid. It is generally considered that the covering bone, the entoplastron, represents the interclavicle and the epiplastra, the dermal or membranous clavicles. Further, it is believed that these structures correspond to the interclavicle and clavicles which are present in fossil Reptiles, such as *Palaeohatteria*.

Otto Jäkel (1918) has described the "plastron" of an archaic type of tortoise (*Stegochelys*) found in the red sandstone strata of Halberstadt. In this, indications of clavicles (epiplastra) and an elongated interclavicle (entoplastron) are present on the dorsal aspect of the plastron which is composed of paired bony plates similar to those of living species. The same author has clearly demonstrated the close relation of the dermal elements of the shoulder girdle (clavicle, interclavicle and cleithrum) with the endoskeletal elements (procoracoid, coracoid and scapula) in the primitive amphibian (*Archegosaurus*) (fig. 5).

A bony shield, the plastron, connected with the shoulder girdle in front, and the bones of the pelvis behind, was present in extinct forms of tortoise which have been found in the Trias and thus in the uppermost Palaeozoic strata.

If this interpretation is correct, namely that the epiplastra of the *Chelonia* are homologous with the membranous clavicles of the extinct *Stegocephalia*, of living and extinct forms of Reptiles and of the Monotremes, it is obvious that independently ossified procoracoids may co-exist with membranous clavicles in the same animal. Consequently the following questions arise more particularly with regard to the bone covering the procoracoid in *Rana*: (1) Is it a dermal bone homologous with the clavicles of the *Stegocephala*? (2) Does it arise from the perichondrium and in the cartilage of the procoracoid, independently of the dermal bone? (3) Is it compound in nature—partly dermal clavicle, partly ossification of procoracoid cartilage?

We may say at once that we believe the interpretation of the homology of the epiplastra and entoplastron of the *Chelonia* with the clavicles and interclavicle of the extinct Amphibia to be correct. The problem to be solved is, therefore, whether the bony plate covering the procoracoid in *Rana* should be called "clavicle" or not. We shall discuss the evidence for and against these views when dealing with the ontogeny of the sternal apparatus in *Rana*. It may be mentioned here, however, that some authors contend that the anterior element of the endoskeletal shoulder girdle of the *Chelonia* does not correspond to the same anterior element in the frog's sternal apparatus, and consider that the anterior bone in the *Chelonia* represents a ventral prolongation of the acromion, and believe that the procoracoid has been suppressed. Moreover,

the ossification of the anterior element (procoracoid or epicoracoid) of Monotremes is regarded as a separated "epicoracoid" bony plate.

(4) *Aves.* Extinct forms, *Archaeopteryx.* Although it is stated that no trace of a sternum was discovered in the well-known Berlin specimen (*Archaeopteryx lithographica*), it is probable that a wide diamond-shaped plate of cartilage was situated between and behind the coracoids and that it articulated in front with the clavicles. The clavicles were fused in the median plane, and lay in front of and between the coraco-scapular elements of the pectoral girdle. *Hesperornis* had a remarkably wide sternum, bowed slightly forwards. It articulated in front with two slender clavicles, the inner ends of which were joined together in the median plane. The sternum also articulated by its antero-lateral borders with the expanded coracoid plates which formed with the sternum a uniformly curved breastplate of extraordinary width. The humerus was ridiculously small, indicating a feeble development of the wing and pectoral muscles. Laterally four or five pairs of ribs articulated directly with the sternum. A similar broad arched sternum was present in the New Zealand moa, *Diornis maximus*, and occurs in the *Apteryx* and other living forms of flightless Birds.

The more primitive cursorial Birds have flat sterna, while the flying Birds possess a keeled sternum which greatly increases the area for the attachment of the powerful pectoral muscles. The sternum is unsegmented. Lillie (1908) described the sternum as originating in the chick from a pair of membranous expansions "formed by the fusion of the distal ends of the four true thoracal ribs"—and states, the "definitive sternum is formed by the concrescence of two lateral halves in the middle line, the concrescence beginning at the anterior end. The concrescence then proceeds posteriorly. Although there are two lateral centres of chondrification, these soon fuse. The various outgrowths of the sternum (episternal process, antero-lateral and abdominal processes) arise as processes of the membranous sternum and do not appear to have independent centres of chondrification. The carina is formed from before backwards as the two sternal bars unite with each other. Thus there is no stage in the ontogenetic development of the sternum of the Carinatae in which the bone exists as a flat median plate."

Our own specimens do not corroborate the first part of this description, but indicate that the paired membranous expansions (mesodermal sternal bars) arise independently of the ribs, and that the union of the tips of the ribs with the sternal bars, as in Reptiles and Mammals, is a secondary event (fig. 7). We are, however, in entire agreement with Lillie's description of the later phases of development of the sternum.

An interesting example of the relation of the ventral ends of the ribs is afforded by the "costal processes" and "pro-ostea" of the mature chick of *Rhea americana*, and the "costal condyles" and "costal processes" of the sternum of *Apteryx*. These were described and figured by W. K. Parker. The costal condyles are lateral outgrowths from the edge of the sternum and

articulate with the ventral ends of the anterior thoracic ribs. A possible explanation of the development of these costal condyles in *Apteryx*, *Rhea* and other Birds in which they are present, is that they arise as a late secondary or adaptive reaction of the sternum to the strain which must occur at the articulation of the rib ends with the edge of the sternum. The "costal processes" or antero-lateral processes, are very similar in appearance to the "costal condyles." These processes project outwards and forwards from the lateral angles of the sternum and thus form two cornua. Each cornu consists of a central core of cartilage which is continuous with that of the sternum and in some Birds, e.g. *Rhea*, the cartilage is strengthened around the base of the

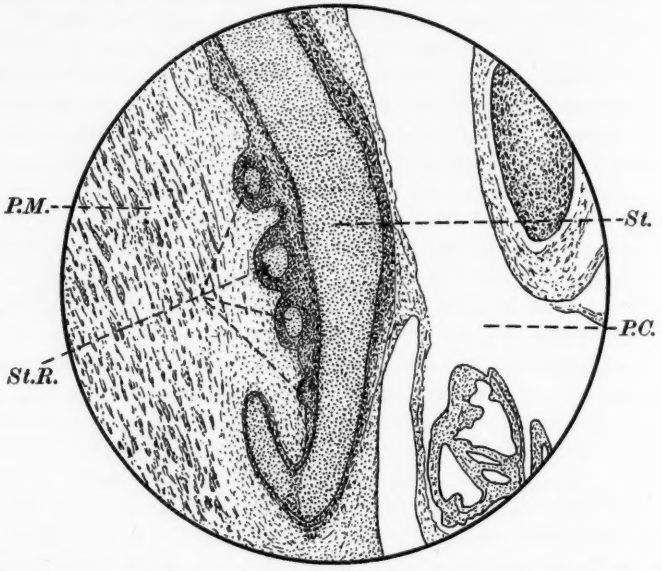


Fig. 7. Section through the junction of the tips of four sternal ribs with the cartilaginous sternal plate; both the sternal and rib cartilage are invested by a thick layer of perichondrium, and are quite independent of each other. Chick embryo (8 days).

cornu with a covering of "ectosteal" bone. The cornua were regarded by Parker as being related to the last of the cervical series of ribs, and in this connection it may be mentioned that they resemble the "sternal cornua" which are sometimes present in cases of cervical rib and rudimentary first thoracic rib in the human subject. Moreover, their presence in *Apteryx* and other Birds suggest that the tips of the last pair of cervical ribs had in the young embryo actually reached the sternum, and that, in later stages of development when the sternum and shoulder girdle are displaced caudalwards, this primary connection had been severed owing to stretching of the intermediate part of the rib which has degenerated leaving the vertebral and sternal

ends connected with the vertebral column and the sternum respectively. Parker's inference of their relation to the last pair of cervical ribs is fully confirmed by the conditions found in the early chick embryo of the common fowl. In a 5½-day embryo, as will be described in detail later, separate centres of procartilage appear for the sternal segments of the last two "cervical" and the four succeeding thoracic ribs. The centres for the sternal segments of the two "cervical" ribs disappear early, whereas those for the sternal segments of the four succeeding ribs extend outwards from the sternal bar along the myosepta and, meeting the tips of the vertebral ribs, complete the costal arches. In *Struthio* and *Rhea*, the dorsal ends of five pairs of sternal ribs join the tips of the corresponding vertebral ribs to complete costal arches, whereas the sternal segments of the first pair retain their connection with the presternum, develop to form costal processes but fail to effect a union with the tip of the vertebral segment of the last cervical rib of the corresponding side.

Whether or not the last pair of cervical ribs in the early chick embryo of *Rhea* extend the whole length between the vertebral column and sternum, as was thought by Goette to have been the case with the last two cervical ribs in the embryo of *Cnemidophorus* (fig. 12), is doubtful. It is more probable that a separate procartilaginous centre appears for the sternal segment of the last cervical rib in *Rhea*, and that this has developed into the costal process, the part of the myoseptum between the costal process and the tip of the vertebral segment of the rib remaining fibrous. If this is the correct interpretation, it is probable that the same explanation holds good for the relation of the cervical ribs to the sternum in other types of animal. The degree of development of the "costal processes" probably depends on the amount of traction exerted upon them by muscles attached to the extremities of the processes. In the human subject, a fibrous band usually connects the "costal process" or "sternal cornu" with the tip of a cervical or rudimentary first thoracic rib. This circumstance, however, does not necessarily imply that the tip of the growing rib ever reached the sternal angle. The fibrous cord is developed from and is in the line of the myoseptum. Later, a cartilaginous centre has appeared in the anterior or ventral end of this myoseptum and has developed into the "costal process" which in some cases becomes ossified to form the so-called "sternal cornu." The latter term is, however, liable to give rise to confusion, since it is not comparable, morphologically, with the "sternal cornua" or "omosterna" of the Rodents.

(5) *Mammalia.* The sternal apparatus of the lowest class of Mammals, the Prototheria, closely resembles that of certain amphibians and Reptiles, e.g. *Bombinator igneus* and *Hemidactylus verucosus*. Membranous clavicles, interclavicle (episternum) and epicoracoids are present in addition to the true sternum.

Segmentation appears for the first time in the Monotremes (fig. 8). The divisional lines lie opposite the attachment of the ribs, and the condition is apparently a secondary adaptation. The presternum is developed in bilateral

halves and receives the ventral ends of two pairs of ribs. The mesosternum consists of three segments which receive the third to the sixth ribs inclusively. In *Echidna* there is a xiphisternum but this is absent in *Ornithorhynchus*. The coracoids are especially large and articulate directly with the presternum. The shoulder girdle and episternum are situated far forwards in adaptation to the burrowing habits of these animals. In *Ornithorhynchus* there are six pairs of sternal ribs. These are connected with the vertebral ribs by intermediate ribs which are imperfectly ossified. Behind the sternum are eight pairs of ribs, the ventral ends of which are expanded into flattened plates. The hinder members of the series overlap those next in front to such an extent as to form two diverging jointed bars which extend backwards from the sternum and give

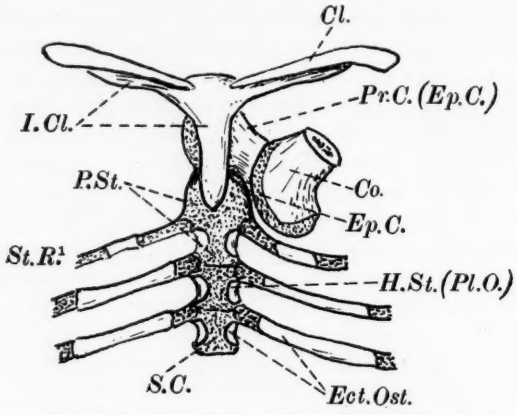


Fig. 8. Sternal apparatus of a very young *Echidna Hystrix*, showing the semilunar "ectosteal plates" covering the lateral aspect of the cartilaginous segments of the sternum, and the tubular sheaths of ectosteal bone covering the sternal ribs. The different segments of the coracoscapular cartilage are not completely separated, but their position is indicated by "ectosteal," or covering plates of bone, which correspond to the scapula, coracoid and procoracoid elements of the Reptilia. (After W. Kitchen Parker.)

stability to this part of the thoracic wall. The arrangement is very similar to that which is present in the more posterior sternal ribs of the crocodiles. Behind the eight pairs of ribs, which are characterised by overlapping ventral expansions, are two pairs of floating ribs which end in free pointed extremities. If the latter are to be regarded as lumbar ribs there are, in *Ornithorhynchus*, sixteen pairs of ribs in all, fourteen thoracic and two lumbar.

In the Marsupialia, the coracoid and clavicle are much more slender than in the Prototheria. The coracoid reaches the presternum in the foetus only (Broom). The clavicle, though nearly always present (the bandicoot being a conspicuous exception), may be incomplete. In the wallaby there are ten pairs of sternal ribs and three pairs of floating ribs. As the fore-limbs and pectoral

girdle are reduced in size, the sternum is also reduced and serves chiefly as an axial bar for the reception and stabilisation of the ventral ends of the ribs.

In the Monodelphia, great variations are met with in the size and form of the sternum, in adaptation to the various habits and requirements of animals belonging to the different orders comprised in this class. In those types in which the fore-limbs are used for burrowing and the pectoral muscles and pectoral girdle are strongly developed, clavicles, epicoracoids and presternum are correspondingly well developed, e.g. in certain of the Edentates, Insectivores and Rodents.

Goette has described, under the name "episternum," two bilateral structures in an embryo mole: (1) the paired "median" episternum lies in front of the presternum and upper part of the mesosternum, and extends forwards for nearly half its length in front of and beyond the manubrium where it articulates with (2) the "lateral" episterna. The right and left medial episterna articulate with each other in the median plane, and the lateral episterna lie, one on each side, between the median episterna and the clavicles. Although the "median episterna" described by Goette resemble in form and position the interclavicle of Reptiles, and it has been shown that in the crocodile, the latter develops from separate bilateral ossific centres (Kalin), yet its origin in the mole as two cartilaginous bars indicates that the "median," like the "lateral" episterna of Goette, correspond to the "sternal cornua" which are present in rodents, e.g. in the mouse. These are usually described as "omosterna" (Reynolds) and undoubtedly belong to the shoulder girdle. The "lateral" episternum of the mole would thus correspond to the articular cartilages between the omosternum and clavicle of the mouse, and probably to the interarticular fibro-cartilage of the sterno-clavicular joint in Man.

In the Sirenia and Cetacea, the clavicle is absent and the coracoid does not reach the sternum. Ribs are numerous, but the anterior members only of the series reach the sternum. In the Balaenoidea, one pair of ribs only articulates with the sternum. In the rorqual, the sternum is a flattened plate of bone, triangular in shape with the apex directed backwards, and presents a notch and small perforation at the anterior border which indicate its bilateral origin.

In the Ungulates and Carnivores, the clavicles and coracoids are incompletely developed or absent and the episternal structures are accordingly reduced or absent. The presternum, mesosternum and xiphisternum are all present. The presternum and mesosternum form a jointed column consisting of separate sternebrae; these receive the sternal extremities of the ribs at the intervals between the segments. A certain amount of mobility is thus provided for the movements of the ribs in respiration, while, at the same time, the ventral ends of the ribs are held in position.

The Rodents have a well-developed clavicle, episternal processes (omosternum) and epicoracoid. The presternum and mesosternum are in the form of a jointed cylindrical column and there is a long xiphisternum which ter-

minates in an expanded cartilaginous plate. In the mouse, seven ribs articulate with the sternum.

In Cheiroptera, in correspondence with the great development of the pectoral muscles, which are used in flight, and the large size of the pectoral girdle, the presternum is also large and is provided with a median keel.

In Primates the sternum is flat. The clavicles are well developed and articulate with a manubrium which varies in size commensurately with the degree of development of the upper limb. It is especially wide and massive in the gorilla. Episternal bones are occasionally present and may be independent or be fused with the manubrium. The manubrium is sometimes fused at an early age with the first piece of the mesosternum: the joint between the thoracic operculum and the body of the sternum lies between the first and second segments of the mesosternum. In Man, this condition is sometimes associated with a rudimentary condition of the first thoracic ribs, as in the cases described by Low and Phillips. It is said to be the normal condition in the gibbon.

Ontogeny of the sternum

The description of the development of the sternum may conveniently be commenced with a reference to the simple stage which is present in the Urodela (fig. 1). A special study of the shoulder girdle and sternum in this sub-class of the Amphibia has been made by Engler, who has demonstrated the presence of the sterno-coracoid membrane in embryos of various types of tailed Amphibia. This membrane which connects the two halves of the definitive shoulder girdle in the adult *Proteus anguineus* has a similar relationship to the right and left halves of the developing shoulder girdles in the embryonic stages of higher types. In the latter it is present in the form of a mesenchymatous band which is gradually invaded by the ventral part of the coraco-scapular cartilage. The scapular, coracoid and procoracoid parts of the common coraco-scapular cartilage become ossified in succession. The bone makes its appearance by means of separate ossific centres which, by enlargement, gradually encroach upon and replace the cartilage; the ossific centres finally coalesce with one another in the region of the glenoid cavity. The different stages in embryonic development of the higher types thus correspond closely to the different stages in evolution, as illustrated in the adult forms of the lower types. Thus in the adult *Proteus*, the scapula only is ossified. In *Megalo-batrachus*, the ossification of the scapula has extended into the ventral part of the shoulder girdle. In *Amphiuma*, the coracoid is also ossified. In *Salamandra*, a procoracoid centre of ossification has appeared, and the three centres unite with one another in the glenoid region and together form a single bony plate. The sternum (fig. 1) in Urodela is wholly cartilaginous and is developed independently of the ribs. In *Salamandra*, as the coracoid cartilages approach each other in their movement towards the median plane, a thickening of the mesoderm occurs along their median borders. This condensation of the mesoderm corresponds to the

mesodermal sternal bars of higher types of Vertebrates. Later, bilateral plates of cartilage are formed in the mesodermal bars. These subsequently fuse with each other in the median plane posteriorly, but remain separate in front where the two plates are connected by a fibrous band which represents the remnant of the sterno-coracoid membrane. Owing to the ingrowth of the coracoids, which overlap each other in the median plane, the sternum is divided anteriorly into a dorsal and a ventral lamina, connected by the above-mentioned remnant of the sterno-coracoid membrane. This stretches between the two plates obliquely in such a way that the plates and membrane in horizontal section present the form of a **Z**-shaped figure, the receding angles of which are occupied by the postero-medial borders of the coracoid cartilages.

A transitional stage between the Urodela and Anura is furnished in the sub-order Aglossa. Thus, in *Pipa Americana*, the procoracoid process of cartilage is almost completely replaced by bone and the epicoracoid cartilages are prolonged forwards into the neck, as a bilateral episternal process. A large coracoid foramen is present between the procoracoid bone and the coracoid. In *Xenopus*, the area corresponding to the coracoid foramen is completely chondrified.

Development of the sternum in Anura

Among the first to study the development of the sternum in the frog were A. K. Parker and A. Goette. More recently a valuable paper has been contributed on the ontogeny of the sternal apparatus in the Anura by de Villiers. Commencing with the development of the sternal apparatus in the frog (*R. temporaria* and *R. esculenta*), Goette shows that at an early stage, when the two halves of the shoulder girdle are widely separated, the ventral part of the coraco-scapular cartilage consists of two limbs, an anterior—the procoracoid, and a posterior—the coracoid, or "metacoracoid." The medial ends of the procoracoid and coracoid are joined by a thickened band of mesoderm. At a later stage, the growing ends of the cartilaginous bars invade this thickened band of mesoderm and, uniting with each other, enclose the coracoid foramen (fig. 9). Subsequently ossification, which takes place at the base of the scapular cartilage, forms the bony part of the scapula; the expanded dorsal segment persists as the suprascapular cartilage. The posterior limb of the ventral segment also becomes ossified and forms the coracoid bone, while the median portion of the cartilage which now connects the coracoid with the procoracoid remains cartilaginous and persists as the epicoracoid cartilage. The right and left halves of the shoulder girdle move towards the median plane and, shortly before the time when they meet and the time when the upper limbs emerge from the opercular chamber, ossification commences on the superficial aspect of the procoracoid cartilages and the anterior margins of the suprascapular cartilages. The two bones formed in this way are described as the "clavicle" and the "cleithrum" respectively. In earlier stages the two bones are separated from the underlying cartilage by a thin layer of fibrous tissue (fig. 4). Gegenbaur

regarded this thin layer of fibrous tissue as an indication of a dermal origin of these bones. A further confirmation of this view has been obtained by de Villiers who has shown that in the commencing ossification of the clavicle in an *Alytes* larva "membrane bone" is deposited in a "cell thickening" in the cutis immediately beneath the epidermis and on the superficial aspect of the procoracoid cartilage. The procoracoid is at this stage situated in the inner wall of the branchial chamber, and the epidermis overlying it forms part of the lining membrane of the chamber. At a later stage, the bone invades the underlying cartilage and medullary spaces appear in the bone thus formed. The histological characters of the developing bone thus show that the ensheathing plates developed over the procoracoid cartilage, and over the anterior border of the scapular cartilage, are primarily dermal in origin notwithstanding the fact that at a later stage they lie beneath a thick layer of

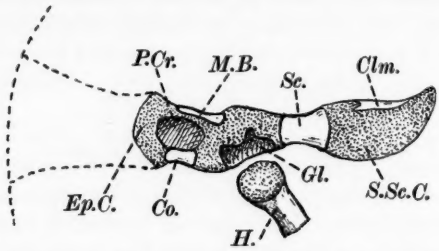


Fig. 9. Left side of pectoral girdle, with humerus in position of tadpole, showing an early stage in the ossification of the coracoid, scapula, and the membrane or covering bones formed on the anterior borders of the procoracoid and suprascapular cartilages. The coracoid and procoracoid cartilages have united with each other so as to enclose the coracoid foramen. The medial plate of cartilage formed by the junction of the coracoid and procoracoid bars, is the epicoracoid cartilage. The two halves of the girdle are still widely separated. The median plane and the area which the girdle will have to traverse in order to reach its definitive position is indicated by the dotted lines. (After W. K. Parker.)

muscle and at a considerable distance from the skin. The ontogenetic development of the bone covering the procoracoid cartilage and scapula thus does not contradict but rather gives support to the view that these bones represent the "clavicle" and "cleithrum" of extinct forms of Amphibia and Reptilia. The old and much-debated question of the true homology of the clavicle in living forms of Amphibia thus appears, in the light of embryological evidence, to be answered by considering the procoracoid cartilage as "zonal" in origin, while the bone covering it, which is a superimposed secondary dermal splint, should alone be described as the true clavicle.

The further development of the median structures of the sternal apparatus in the tadpole may now be considered. Soon after the two halves of the pectoral girdle have met, a median band of procartilage, which lies between the two epicoracoid cartilages (fig. 4), is found to be present. This median band extends forwards in the median plane between the sterno-hyoid and

coraco-radialis muscles and backwards between the rectus abdominis muscles. The intermediate part of this procartilage which lies between the epicoracoids disappears to a large extent, being absorbed as the epicoracoids fuse with each other in the median plane. The anterior prolongation gives rise to the episternal process (omosternum), the basal part of which becomes ossified while its anterior part remains cartilaginous (figs. 10, 11). The posterior prolongation of the procartilage, which extends backwards into the linea alba, gives rise to the sternum. It is probable, however, that a backward extension of each epicoracoid cartilage also contributes to the formation of the sternum (fig. 11, E, F), and that these take part in the formation of the basal bony segment, whereas the cartilaginous extremity, which expands to form the terminal disc, is wholly derived from the procartilage. This procartilage, which gives origin to the sternum, is laid down in the tissue formed by the fusion of the bilateral plates of condensed mesoderm which previously joined the inner ends of the procoracoid and coracoid cartilages; it appears to be similar in nature to the median sternal plate which is formed by the union of the right and left sternal plates in the Amniota.

Development of the sternum in Reptilia

The ontogeny of the shoulder girdle in the Lacertilia has been studied by a considerable number of workers, among whom may be mentioned Bogoljubsky, Gegenbaur, Goette, and Mary Juhn. Selecting *L. muralis* as a type form we may first allude to the principal features of the sternal apparatus in the adult animal. The sternum consists of a rhomboidal or lozenge-shaped plate, with an oval opening situated in the median plane and a little below its centre. Three pairs of sternal ribs articulate with the postero-lateral borders of the sternum, and its caudal end is prolonged backwards in the form of two sternal cornua with which the fourth and fifth pairs of sternal ribs articulate. None of the cervical ribs reach the sternum. Each antero-lateral border of the sternum is thickened where it articulates, for nearly its whole length, with the similarly thickened postero-medial border of the coracoid element. Obviously one of the principal functions of the sternum is to afford a fulcrum for the support of the shoulder girdle. A large fenestra is present in the coracoid segment of the pectoral girdle of which the antero-lateral boundary is formed by cartilage representing the procoracoid, while the postero-medial and medial boundaries represent the coracoid and epicoracoid cartilages respectively. Superficially disposed to the coracoidal elements and to the sternum are the cruciform interclavicle (episternum) and the clavicles. The expanded inner ends of the two clavicles, which are perforated by an oval opening, articulate with the short upper limb of the cross formed by the interclavicle, while their outer ends overlap the dorsal extremity of the scapula of the corresponding side on its ventral aspect and close to its articulation with the suprascapular cartilage. The sternum consists of calcified cartilage only, whereas the clavicles and interclavicle are from their first appearance formed of true bone.

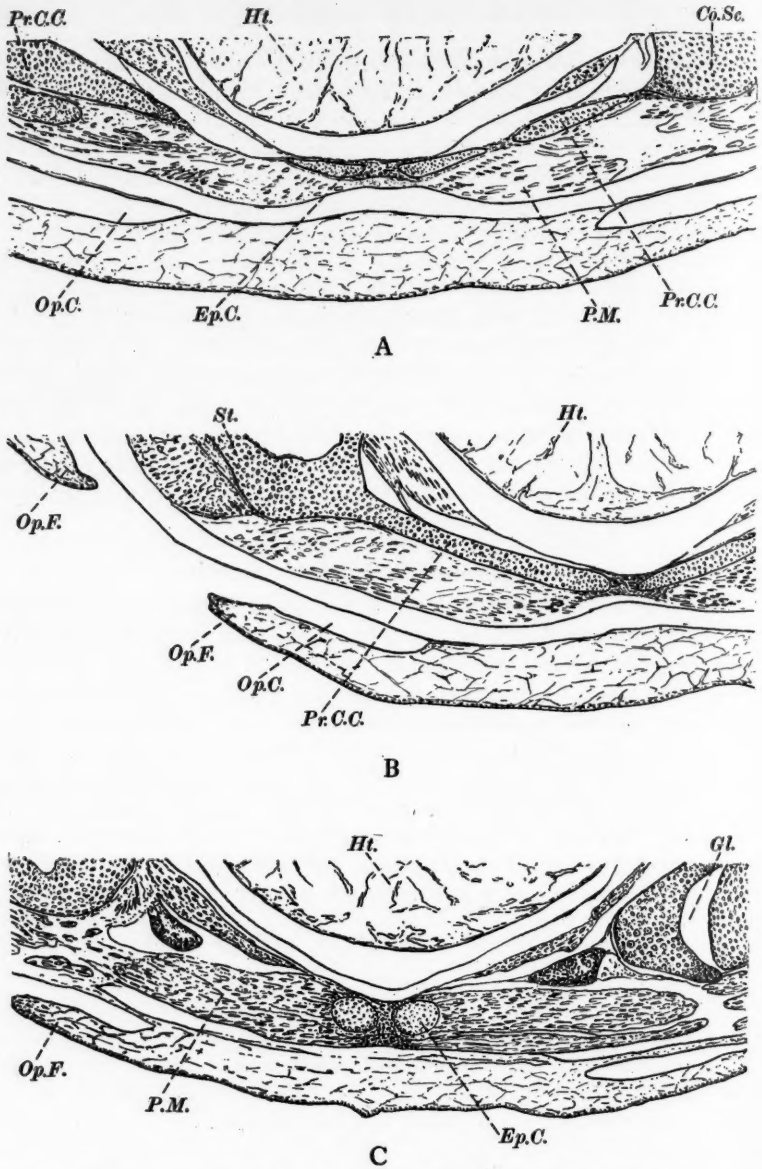
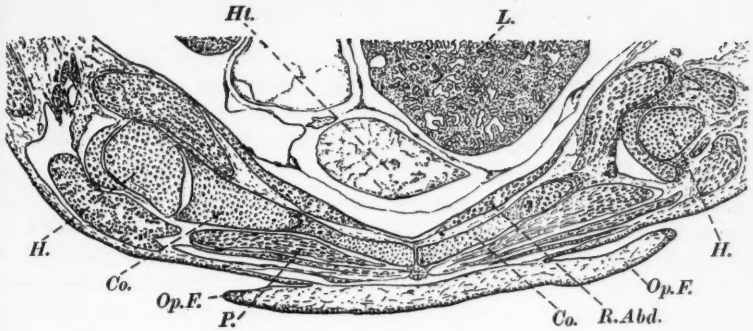


Fig. 10. Transverse sections through a tadpole of *Rana temporaria*. A. Through the procoracoid cartilages and base of the episternal process. B. Through the procoracoid cartilages, showing, on the right side, continuity of the procoracoid with the scapular cartilage. C. The epicoracoid cartilages which form the inner boundaries of the large coracoid foramina have met in the median plane, but are separated by an intervening plate of procartilage. The epicoracoid cartilage is continuous in front with the procoracoid bar, and behind with the coracoid, and is formed by the union of these two with each other.



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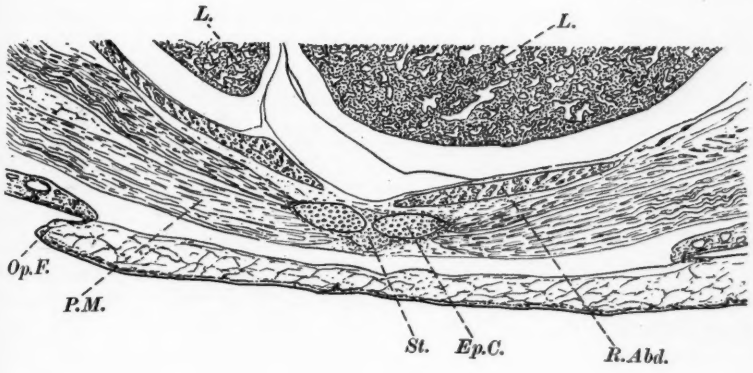

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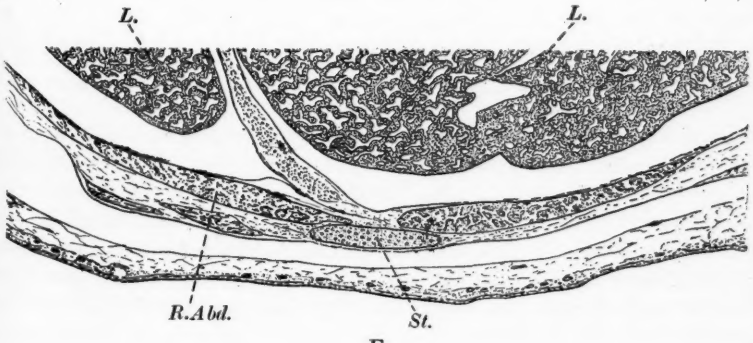

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Fig. 11. Transverse sections through a tadpole of *Rana temporaria*. D. Section through the coracoid bars and glenoid cavities, showing commencing ossification of the former. E. Section passing behind the coracoid bars, showing a backward extension of the cartilage into the region of the sternum. F. Section through the posterior end of the sternum, which is formed here by an extension backward of the procartilage of the sternal bars into the linea alba.

The earliest stages of development of *Lacerta*, investigated by Mary Juhn in 1923, were studied in embryos of 9·5 mm. and 10 mm. in length. The two sternal plates were found to be widely separated, triangular in form, and consisted of dense mesoderm. The coraco-scapular cartilage was in the condition of quite young procartilage and the clavicles were represented on each side by a small bony rod. In the 9·5 mm. embryo, she describes the first ribs as being continuous with the lateral angle of the sternal plate which spreads out in a ventro-medial direction from this point. In the 10 mm. embryo, three ribs were found to be in continuity with the sternal plate; a joint was also present between the procartilage of the coracoid and the sternal plate. The ossification of the clavicle was well advanced and extended for nearly its whole length.

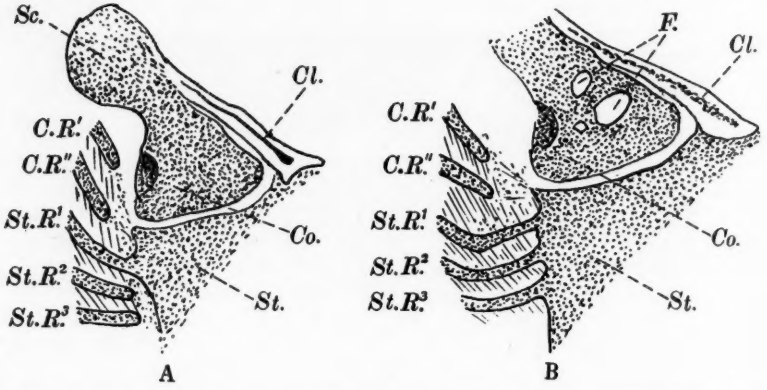

Fig. 12. Two stages in the development of the sternum and ribs of *Cnemidophorus*, a North American lizard. A. The earlier stage illustrates a phase in which only one rib, the first thoracic, has reached the sternum; in B, the second and third thoracic ribs have also joined the sternum. The last two cervical ribs are connected with the supero-lateral angle of the sternum by fibrous bands. These remain unconnected with the sternum in the adult animal.

In two specimens of *Cnemidophorus* (fig. 12), described by Goette, of about the same stages of development as the embryos studied by Mary Juhn, the earlier specimen showed that the junction of the first sternal rib with the sternal plate had already been established, while the second and third ribs were separated by a considerable interval from the sternal plate. In the older specimen three sternal ribs had joined the sternum. The joint between the coracoid and sternum was also present in both these specimens and ossification of the clavicles had commenced. Two fenestrae were present in the coraco-scapular cartilage of the older specimen. In close relation with the lateral angle of the sternal plate, and connected with this by some fibrillar strands of mesenchyme, were the last two cervical ribs. Goette believed that the two cervical ribs, shown in the figures, had, at an earlier stage of development, been directly connected with the lateral angle of the sternum and that

the primary connection had been lost. It is more probable, however, that the fibrous connections shown in the figures represent the ventral ends of mesenchymatous myosepta which had not been invaded by the growing ends of the cervical ribs, and also that in the course of further development, as the pectoral girdle and sternal plates moved towards the median plane and caudally, the distance between the sternum and the cervical ribs would have been increased. In other words, the distal ends of the cervical ribs would have been left free in the body wall because the rate of growth of the cervical ribs would not have kept pace with that of the sternal plates, and their growth would have become arrested before the ribs had reached the sternal plate. On the other hand, the rate of growth of the five succeeding ribs was sufficiently rapid for these to have effected a junction with the sternal plates, and this once having been established, their growth in length would keep pace with and would possibly act as a causal factor of the movement of the sternal plates towards the median plane.

This view of the relation of the cervical and sternal ribs to the sternal plates is supported by the evidence brought forward by Bogoljubsky who, employing embryos of *L. muralis*, *L. vivipara* and *L. agilis*, showed that in the early stages of *L. muralis*, in which the sternal plates and ventral ends of the ribs consist of dense mesoderm, there is a distinct interval occupied by loose fibrillar mesenchyme between the growing ends of the upper three sternal ribs and the sternal plates (figs. 18*a* and 18*b* of his Plate XVII). In a slightly later stage (figs. 23*a*, *b*, and *c* of Plate XVIII), he shows that the space between the first three sternal ribs and the sternal plate is lessened, but there is still a distinct interval occupied by fibrillar embryonal mesenchyme. The younger of these two embryos also shows the early primary continuity of the coracoid rudiment with the sternal plate (fig. 19, Plate XVIII). In his sections 34*a*, Plate XVIII and 34*b*, Plate XIX, a closer approximation of the ventral ends of the ribs with the sternal plates is seen, a union of the dense mesenchyme around the sternal ends of the first three ribs with the sternal plates having already taken place. The first sternal ribs are more closely united with the sternum than are the second, and the second more closely than the third. The fourth and fifth pairs of sternal ribs join the prolongations of the sternal plates which constitute the sternal cornua and do not fuse with each other in the median plane.

The clavicles and right and left moieties of the interclavicle are developed as dermal bones appearing first as condensations of the mesoderm immediately beneath the skin. The ossification takes place very early and exhibits the mode of development characteristic of membrane bone, without any preformation of cartilage. Bogoljubsky concludes that the sternal rudiments are developed independently without any participation of the ribs. The fusion of the ribs with the sternum takes place in serial order, commencing with the first, and is completed before the sternal plates unite with each other. The shoulder girdle on each side is represented in the early stages by a uniform layer of embryonal connective tissue which gives rise to the pars scapularis,

pars coracoidea and in part to the pars clavicularis. Very early, the cranial part of the shoulder girdle, which is in immediate relation with the epidermis, is separated from the remaining part of the girdle and only remains connected with it at its proximal or dorsal end. This cranial part, which consists of embryonic connective tissue, becomes separated from the skin, ossifies immediately without any preformation of cartilage and gives rise to the clavicle. The prescapula, procoracoid and epicoracoid are differentiated secondarily from the pars coracoidea by a process of resorption, first of the mesenchyme and then of the cartilage, whereby the fenestrae or fontanelles I and II appear as intervals distinguishing the prescapula, procoracoid and epicoracoid from one another. Bogoljubsky considers that the connection of the clavicles with the sternum in higher forms may be explained by assuming that the dermal clavicles, primitively requiring a support for their inner ends, sent out processes which, primarily bilateral, became fused together to form the interclavicle. This, as occurs in the ontogeny of the Lacertilia, assumed the form of a raft and as such is present in Palaeohatteria and other fossil Reptiles. In the course of phylogeny, the clavicle has separated from the skin and the primitive tendency for the clavicle to obtain support from the interclavicle is transferred from the interclavicle to other elements of the shoulder girdle. This results in the reduction of the interclavicle and approximation of the clavicles to the sternum; ultimately the clavicles articulate with the sternum as occurs in higher types, e.g. many of the Mammalia in which the interclavicle has almost completely disappeared. Further, a disappearance of the clavicles, such as occurs in the crocodiles, is accompanied by a corresponding reduction of the interclavicle.

Van Geldern, working on embryos of *L. agilis*, came to the same general conclusion with regard to the relation of the ribs to the sternum as Bogoljubsky, namely, that the union of the ribs with the sternal plates occurs secondarily and does not eventuate until the ribs have reached the prochondral stage. Van Geldern regards the sternal plates as first appearing in the form of isolated thickenings of the mesenchyme in the side of the body wall. Mary Juhn criticises these findings of Bogoljubsky and Van Geldern on the basis of the condition in her specimens, which she considers to represent younger stages, in which the ribs were continuous with the sternal plates at the first appearance of the latter. She implies, however, a secondary union of the ribs of the thirteenth vertebra in stage III, when she states that the ribs of the thirteenth vertebra have not yet reached the sternal plates, although the paired prolongations of the latter are already formed. According to Bogoljubsky, the sternal cornua are prolonged backwards, and the ribs of the twelfth and thirteenth vertebrae (fourth and fifth sternal ribs), join the sternal cornua secondarily in a manner similar to the first, second and third ribs.

Development of the sternal apparatus in Crocodilia

The ontogeny of the sternum has been studied in the Crocodilia by J. A. Kalin, and more particularly in *Alligator mississipiensis*. He found a well-developed bony coracoid in a young animal of this species. The epicoracoid is represented by a thin cartilaginous lamella which appears as an unossified epiphysis occupying the interval between the bony coracoid and the calcified presternum. The clavicles are absent and the interclavicle is a median bar of bone which projects for about half its length beyond the anterior border of the presternum, whereas the caudal half, which tapers to a point, lies on a groove ventral to the anterior half of the presternum, a flat pentagonal plate which is directly continuous behind with the mesosternum. The presternum articulates by its antero-lateral borders with the coracoids, thin plates of cartilage representing the epicoracoid intervening. The first two pairs of sternal ribs join the postero-lateral borders of the presternum directly without any appearance of a joint. The narrow mesosternum is continuous with the presternum in front and with the xiphisternal processes or sternal cornua behind. Laterally it receives the third to the sixth pairs of sternal ribs. The seventh rib joins the sternal cornu of the corresponding side directly. The xiphisternum consists of two diverging cornua which are directly continuous in front with the mesosternum without any indication of a joint between them. In the adult animal the sternum consists of calcified cartilage, whereas the interclavicle is composed of true bone.

In the earliest stages of development the first appearance of the sternum is indicated by paired streaks of condensed mesoderm which arise in the lateral part of the body wall in the thoracic region. These are continued forwards on each side without any interruption into the mesodermal rudiment of the coracoid. It is significant that, at this stage, none of the rib rudiments is continuous with the mesodermal sternal bar. The mesodermal sternal bars are at first widely separated. Later, the sternal bars and coracoids become converted into procartilage, and the former become separated from the coracoids. Subsequently, six pairs of procartilaginous sternal ribs join the lateral borders of the sternal bars. In the later stages, fusion of the sternal bars takes place in front, proceeds from before backwards, and thus the presternum and mesosternum are formed. Behind, the sternal bars fail to fuse; they remain separate and persist as two diverging sternal cornua. The pars sternalis of the first thoracic rib on each side is, at first, in close relation with the sternal bar and appears to be developed independently of the pars vertebralis. Kalin considers that if the linea alba is to be regarded as a product of the transverse skeletogenous septa, the morphological difference between sternum and ribs is not so significant as is usually supposed, for both ribs and sternum occur as chondrifications of homologous skeletal septa.

The interclavicle of the alligator first appears as an unpaired rudiment. The ossification of this is, however, bilateral, indicating its primary origin as

a paired structure. It is originally independent of the sternum and is a covering bone which only secondarily comes into relation with the sternum.

Ontogeny of the sternum in Birds

Although considerable attention has been directed towards the development of other skeletal structures in Birds, the development of the sternum, more especially as regards the early mesodermal stages, appears to have been neglected.

There are, however, two papers dealing with the development of the sternum which have been published recently, one by W. Kopfli in 1918, and another by J. H. Hommes in 1921. Kopfli observed a condensation of embryonal cells at the distal ends of the thoracic ribs in a chick embryo of the sixth day (143 hours). He regarded this condensation as the forerunner of the sternum. His conception of the relation of the ribs to the sternal bars will be best conveyed by a verbatim quotation of his description: "Es (sternum) besteht mithin aus zwei Bändern, den sog. Sternalleisten, die ventral an den Rippen zur Ausbildung gelangen, indem sich das Rippengewebe an den freien Enden derselben ausbreitet. Dadurch treten die einzelnen Rippen miteinander in gewebliche Verbindung. Auf diese Weise wird ein leistenformiges Gebilde geschaffen. Anfangs stehen die beiden Leisten noch weit auseinander, sie werden erst durch eigenes Wachstum in der ventralen Richtung und durch Langenausdehnung der Rippen medialwärts verschoben und einander genähert. Das Brustbein verdankt seine Entstehung sonach den Rippen." Kopfli therefore regards the sternum as owing its origin to the ribs and believes that the sternal bars are formed by the extension of tissue from the free ends of the ribs into the connective tissue of the two sternal bands and that the ribs themselves eventually give rise to a bar-like structure. It is obvious, however, that he admits the existence of the two "bands" which he expressly states lie ventral to the ribs, before the "costal tissue" at their free ends spreads out into the bands. The bands themselves are, therefore, according to this description, developed prior to and independently of the ribs. This is in accordance with the conditions which we have observed in our own material. The participation of the ribs in the formation of the sternal bars which he describes in the following sentences is, however, completely negatived by the appearances which are seen in later stages of our specimens. These show that the cartilaginous sternal bars arise by an independent chondrification of the early mesodermal "bands," and that the tips of the ribs are at no period of development continuous with the tissue of the sternal bars, but are separated in the late procartilaginous and cartilaginous stages by a dense mesodermal sheath or perichondrium (fig. 7). In the early procartilaginous stage (5½ days), the ventral ends of the sternal ribs are sharply defined and are quite distinct from the bar of condensed mesoderm from which the sternal plate is derived (fig. 13).

In a 5½-day chick embryo, we find that the mesodermal bars appear in the anterior thoracic region opposite the limb buds in the same situation as in Amphibia, Reptiles and Mammals. These bars are at first separated by the whole width of the body cavity and have the same form and muscular relations as in other classes of animals. The coracoid, scapula and vertebral ribs are in an early stage of procartilaginous formation. Between the ventral end of the pars vertebralis of a thoracic rib and the sternal bar is a band of fibrillar mesenchyme which represents the sternal segment of a myoseptum. These bands show a definite condensation of nuclei and loss of fibrillar structure at their ventral ends where they impinge against the sternal bar. The condensations (fig. 13) have the appearance of an early stage of procartilage. Their

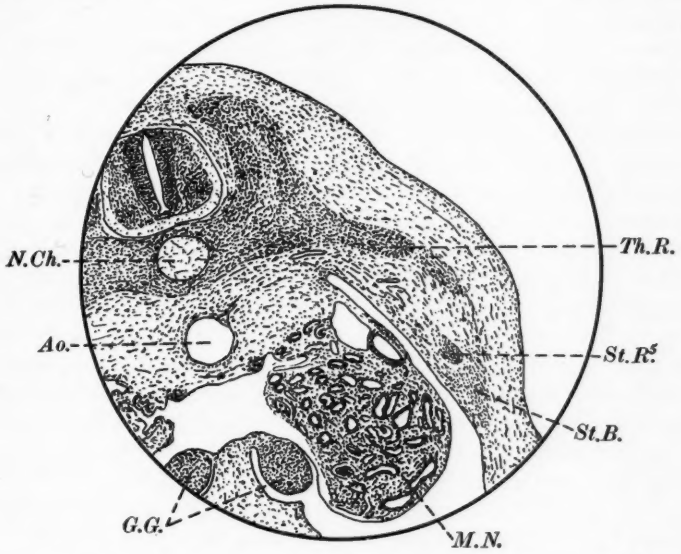

Fig. 13. Transverse section through a 5½-day chick embryo, traversing the sternal region, gonads and mesonephros. It shows a mesodermal sternal bar, and one of the separate centres of procartilage for a sternal rib.

ventral extremities present a rounded contour which is sharply marked off from the sternal bar; at this stage the latter consists of condensed non-fibrillar mesoderm and does not stain so deeply with haematoxylin as do the rib elements. If the rib elements are traced dorsally the procartilage gradually merges into the fibrillar mesenchyme of the intermuscular septum. These condensations of the mesoderm at the sternal ends of the intermuscular septa indicate the first appearance of a separate centre of chondrification for the pars sternalis of each thoracic rib, similar to that which Kalin has described in the alligator. It is also clear that the sternal part of the rib, which is laid down in

the myoseptum, is developed quite independently of the sternal bar, and that, although closely related, the sternal bar is not as is sometimes stated a product of the ribs. In a 7-day chick embryo, the procartilage is seen to be largely replaced by cartilage and that syndesmodial joints are present between the vertebral and sternal segments of the ribs, and also between the latter and the "pars costalis" of the sternum. The same condition, but at a much more advanced stage, is present in an 8-day chick embryo (fig. 7), in which it will be seen that a thick layer of perichondrium intervenes between the ventral ends of the sternal ribs and the cartilaginous costal segment of the sternum. At this stage the two halves of the sternum have united in the median plane and the formation of the keel has commenced. The sternal bars, after passing through a procartilaginous stage, are chondrified in a uniform manner without segmentation. The various processes of the sternum are formed by an extension of the process of condensation from the primary mesodermal sternal bar, later by an extension of a continuous process of chondrification into the secondary processes. There is no indication of serial segmentation in the development of the sternum in Birds, such as might be expected to occur if the sternum were formed by the fusion of the ventral ends of the thoracic ribs. In short, sternal bars in Birds are developed independently of the ribs, as in the Amphibia and Reptiles, and in the earliest stages of mesodermal condensation are very closely associated with the inner ends of the coracoids.

The number of these procartilage centres of the sternal ribs in the 5½-day chick embryo is six on each side. The upper two, which are less distinct than the succeeding four, probably degenerate early. In an 8-day chick embryo (fig. 7), the tips of four ribs only articulate with the sternum, though an indication of the second is still present. In the adult fowl there are four pairs of ribs which join the sternum. The last two pairs of the cervical series are short and fail to reach the sternum; they consist of the vertebral segments of a rib only, the sternal segments having degenerated at an early period of development, namely between the stage when they first appear as a procartilaginous rudiment, 5½ days, and the eighth or ninth day, when their existence is only indicated by slight swellings on the lateral border of the sternum. In the ostriches and emus, five pairs of sternal ribs join the sternum in the adult bird. The sternal segments of the first pair are represented by the costal cornua to which reference has previously been made.

Ontogeny of the sternum in Mammals

The condition of the sternum in a young *Echidna* (fig. 8) has already been alluded to (p. 528), and the very close correspondence of the shoulder girdle and sternum of Monotremes to certain forms of primitive Reptiles was emphasised. This resemblance is well seen in a comparison of the pectoral girdle and sternum of the Anomodont *Dicynodon* (Broom) with those of the *Echidna*.

In the Marsupialia, a type of shoulder girdle and sternum is present which is transitional between that of the Monotremes and that of the placental Mammals. The resemblance is more apparent when the foetal condition of the girdle is examined, as was done by Broom in 1895, in a very young specimen of *Trichosurus* taken from the pouch. At this stage of development, the coracoid extends ventrally as far as the sternum to which it is joined close to the articulation of the first pair of ribs with the sternum. During later growth, the inner end of the coracoid atrophies and in the adult animal the primary connection with the sternum is lost. The clavicles are ossified early in mesenchyme,

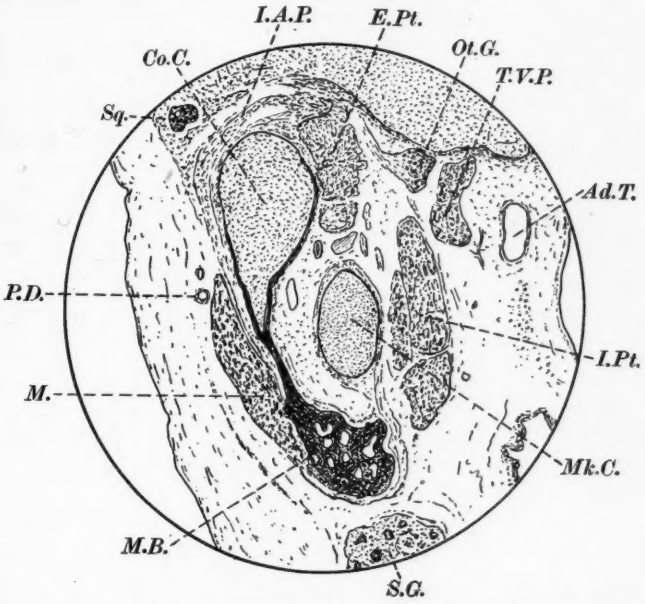

Fig. 14. Coronal section through the temporomandibular articulation of a human embryo (6·5 cm.), showing the relations of the membrane bone of the lower jaw to Meckel's cartilage, and the secondary cartilage formed in the condylar process of the mandible.

before any cartilage is found in them, and Broom considers that, in the Monotremes and Marsupialia, the clavicle is a true membrane bone without any trace in it of the procoracoid and, further, that the cartilage cells found in the developing clavicle in higher Mammals are of no more significance than the cartilage found at the angle and in the coronoid process of the mandible in many Mammals.

At the inner ends of the clavicles in *Trichosurus* are two small skeletal elements which arise in cartilage. They are frequently described as the "omosternal" bones and probably represent remnants either of the epicoracoid or

of the procoracoid cartilage. The clavicles are ossified early in mesenchyme before any cartilage has appeared. With reference to this statement it may be of some importance, in deciding whether a bone is membranous in origin or belongs to the endoskeleton, to note the type of cartilage which is present and also to notice whether it is the cartilage or the bone which develops first. In the human subject a very considerable difference exists between the character of the cartilage which appears secondarily at the angle, condylar and coronoid processes of the lower jaw, and that of Meckel's cartilage which represents the primary skeletal basis of the mandible (fig. 14). The same principles hold good

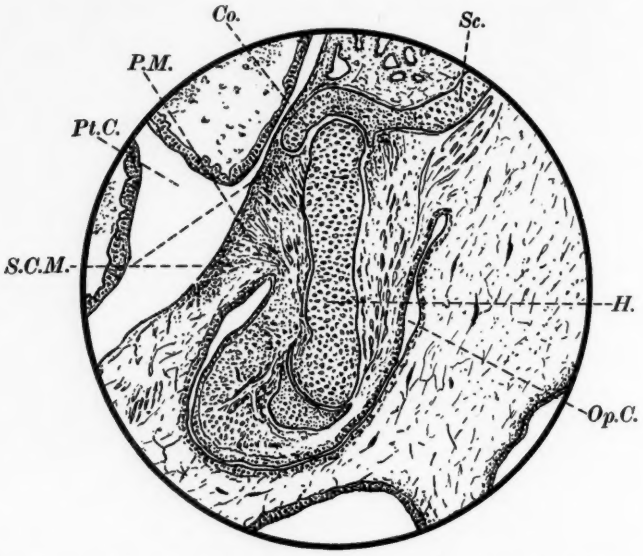

Fig. 15. Transverse section through tadpole (2·4 cm., *Rana temporaria*), showing a condensation of mesoderm, which gives origin to the pectoral group of muscles and represents the sternal bar of amniotes. The two sternal bars are separated by the whole width of the abdominal cavity.

in other situations where dermal and endoskeletal bones are associated and should be carefully considered in determining the nature of such bones as the clavicle and the sternum with its related skeletal structures, such as the "episternal" bones. We agree with Broom in thinking that histological characters such as the presence of cartilage cells in developing bone should not be considered alone; ontogeny and phylogeny should be considered together. Some bones appear to represent a transitional stage between a purely "membrane" bone and an endoskeletal or cartilaginous bone. From the standpoint of comparative anatomy we believe that such bones furnish important clues

to the wider problem of the general relation of the endoskeleton to the exoskeleton.

In considering the ontogeny of the sternum in placental Mammals, we are confronted with great variations in detail, more especially with regard to the relations of the clavicles and coracoids and with regard to the number of sternal ribs. The general principles of the development of the sternum, however, coincide with that found in lower Vertebrates, namely the appearance of two mesodermal bars, placed bilaterally in the thoracic region and separated originally by the whole width of the abdominal cavity or of the heart and

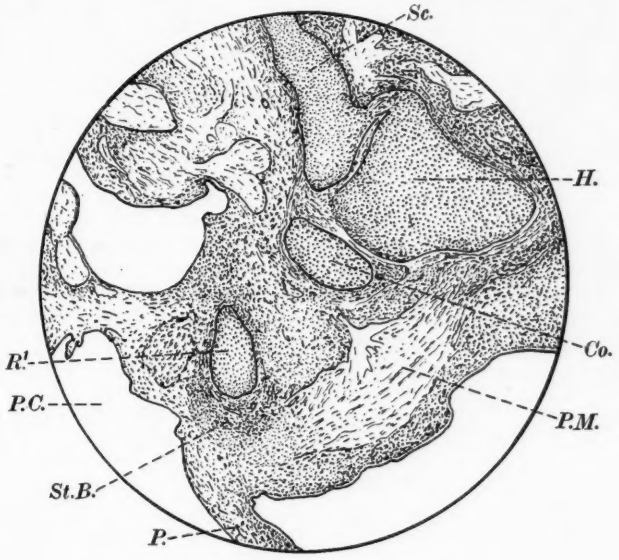

Fig. 16. Transverse section through a human embryo (16 mm.), showing the relation of the sternal bar to the tip of the first rib, and pectoralis major. The space lying medial to the sternal bar and rib is the pericardial cavity. Compare figs. 3, 6 and 13.

pericardium (figs. 3, 6, 13, 15 and 16). Each sternal bar is closely associated with the shoulder girdle, with the ribs and with the origin of the pectoral muscles (figs. 16, 17), but does not appear to be derived from any of them. As the associated structures move towards the median plane the sternal bars move with them and, on reaching the middle line, they fuse with each other as is the case in lower classes of Vertebrates (figs. 17, 18). A median element, possibly representing the interclavicle, is present in some forms and bilateral episternal structures which probably correspond to vestiges of the epicoracoid and procoracoid cartilages may be present (figs. 19, 20). These may fuse with the presternum, or form a connecting bone or cartilage between it and the clavicle.

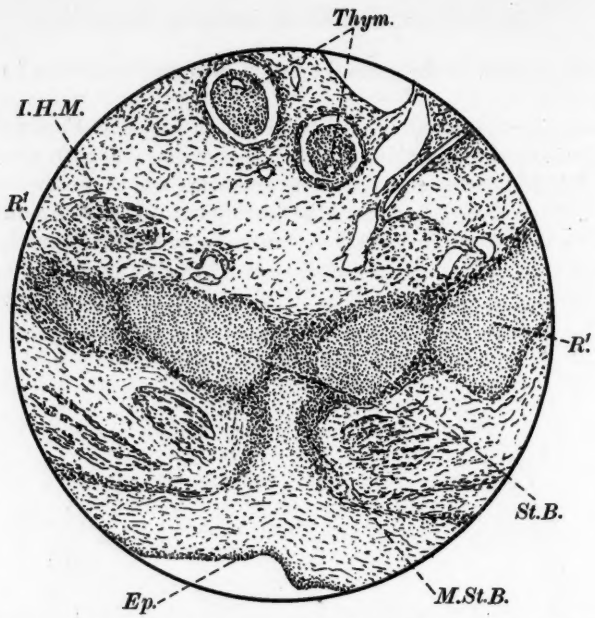

Fig. 17. Transverse section through a human embryo (22 mm.), showing the relation of the ventral ends of the first rib cartilages with the cartilaginous sternal bars. Note the thick perichondrial layer which, at this stage, separates the ribs from the sternum.

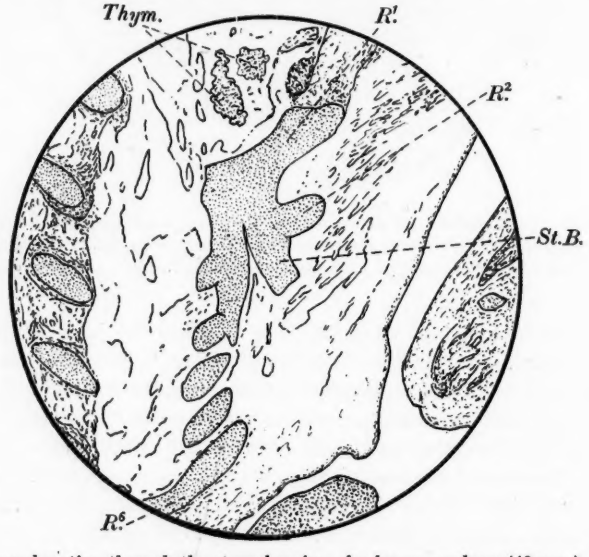

Fig. 18. Coronal section through the sternal region of a human embryo (40 mm.), showing the cartilaginous sternal bars joined above, diverging below. The cartilage of the first rib is completely joined with the presternum. There is a slight indication of the junction of the second left and third right ribs with the sternal bars.

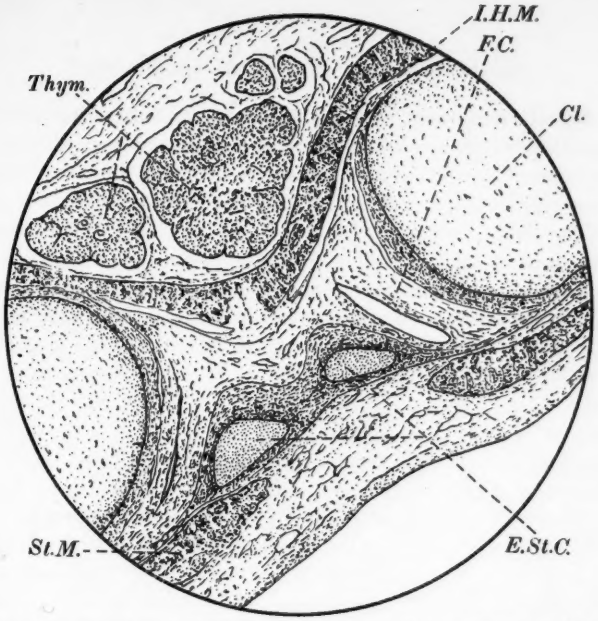

Fig. 19. Horizontal section through the sterno-clavicular articulations of a 45 mm. human embryo. The interarticular disc is fibrous in character. The episternal cartilages are continuous below with the cartilage of the manubrium.

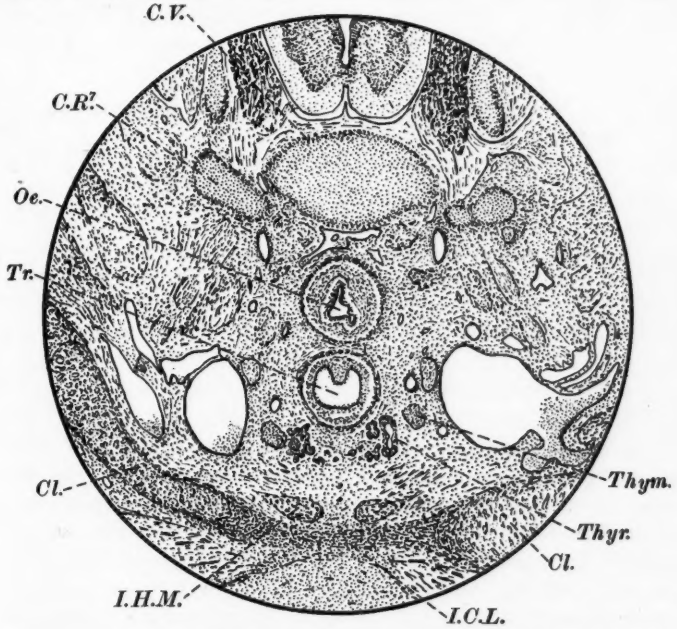

Fig. 20. Transverse section through the lower cervical region of a human embryo of 22 mm. length, showing the extent to which the cartilaginous seventh cervical rib is normally developed, and also the relations of the inner ends of the clavicles. Ossification of the clavicles is well advanced. They are connected by a band of condensed mesoderm, which gives rise to the interclavicular ligament. No episternal cartilages are present in this embryo.

The "episternal" structures, clavicles and coracoidal elements vary greatly in their degree of development, but there is a tendency towards regression of these structures. The episternal structures, more especially, tend to disappear. The mode of chondrification of the sternal bars is also variable both as regards the period at which it occurs and its relationship to that of the ribs.

In some animals such as the pig (Kravetz) and rat, well-developed cartilaginous hemisternebrae appear in the sternal bars between the ends of the ribs. In others, the appearance of cartilage is a more uniform and general change and is comparable with that which occurs in Birds and Reptiles. The change

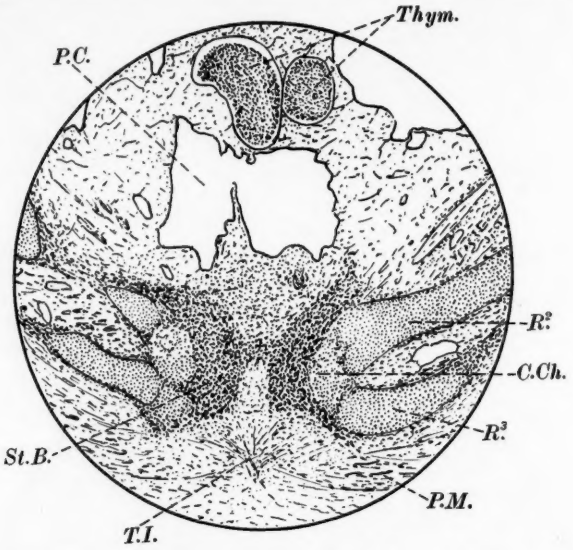

Fig. 21. Transverse section through a human embryo (22 mm.), showing centres of chondrification between the ventral ends of adjacent ribs, and on the lateral margin of the sternal bars. The latter are now near the median plane and are connected by a transverse bar of condensed mesoderm at the level of the ventral ends of the second ribs.

from procartilage into cartilage is often so rapid that it is necessary to study a very closely graded series of specimens in order to determine the exact conditions. In the human subject, for instance (figs. 18, 21), and *Cavia cobaya* (fig. 28), the cartilaginous sternal plates soon become uniform and no traces of the formation of the cartilage from separate centres or of serial segmentation are visible. The rib cartilages are also for a period directly continuous with the cartilage of the sternum, only a faint trace of their junction being discernible (figs. 18, 25 E and 29). Ossification varies greatly in different species, both as regards its time of onset and the position of the separate centres. In some specimens, ossification is well advanced at the time of birth, in others, e.g. the

mouse, it has only just commenced. The general conclusions of A. Melville Paterson with regard to the phylogeny and ontogeny of the sternum, which were founded on his observations on rat, mouse and human embryos, are thus confirmed by subsequent workers. Paterson contended that the sternum arises from a mesodermal condensation in close association with the shoulder girdle and that its further development is accompanied by the appearance of separate centres of chondrification in the two mesodermal bars which extend caudally from the primary median condensation. The centres of chondrification, which appear in the mesodermal sternal bars between the ventral ends of the costal cartilages enlarge, meet and fuse with each other to form right and left cartilaginous sternal bars, each of which is joined along its outer edge by the corresponding series of rib cartilages. Although it is evident that Paterson neither saw nor described the early stage in which the mesodermal costal bars have not yet joined the mesodermal sternal bars, he correctly inferred that the junction of the ribs with the sternum was secondary, and further, that its development was primarily more closely associated with the shoulder girdle than with the ribs. Our own observations have been made chiefly on the development of the sternum in the mouse, rabbit and human embryos. The series of mouse embryos has been lent to us for this purpose by Prof. Mackinnon and Mr Ben Dawes, B.Sc., from the Zoological Department of King's College, London, and we wish to express our indebtedness to them, and also to Mr E. McLellan and Mr J. Taliat for their assistance in the construction of the two models of which the following is a description.

Model of the episternal region of a mouse embryo by Jacob Taliat, F.R.C.S.

The model (fig. 22) consists of the upper part of the sternum, the first three pairs of ribs, the episternal region and the left clavicle. The embryo was one of a closely graded series of mouse embryos kindly lent to us by Mr Ben Dawes. It measured 8·5 mm. in length and showed the important transitional stage between the formation of procartilage and cartilage.

The sections were made transversely and were 10 mm. in thickness. Outline drawings of the parts to be modelled were made from each section at a magnification of 100 diameters and these were rolled on wax plates 1 mm. in thickness. The drawings represented the outer limits of the perichondrial or periosteal tissue and also the outline of the central core of cartilage or bone. In addition to the skeletal structures, the oesophagus and entodermal lining of the trachea were included. The centre of the former was used in the reconstruction of a dorsal axial line and a point in the mid-ventral region of each section was employed in the reconstruction of a ventral axial line.

The left clavicle, which was completely reproduced, shows a constriction indicating its division into an upper and a lower segment. The section shows that the upper and outer segment is more advanced in its development than the lower and inner; ossification has already commenced in the former but

not in the latter. The lower and inner part shows a central core of cartilage. The whole clavicle is invested by a thick covering of dense periosteal and perichondrial mesenchyme which is continuous medially with the sternal cornu of the corresponding side. Each cornu is, however, separated by a well defined groove from the sternal end of the clavicle and terminates cranially in a free rounded process.

Between the tips of the sternal cornua is a curved band of dense mesenchyme which is the precursor of the interclavicular ligament. The sternal cornua contain a central core of procartilage. A small nodule of procartilage is also present in the median plane between the clavicles and is embedded in a mass of dense mesoderm which appears triangular on section. The apex of the triangular mass is directed ventrally and is continuous caudally with a median fibrous

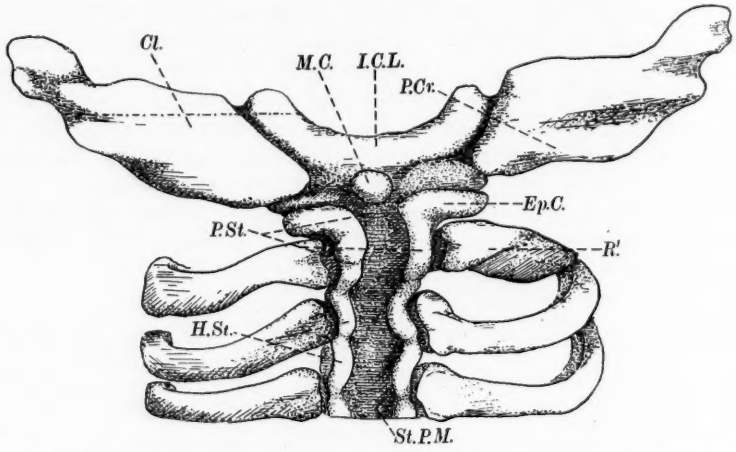

Fig. 22. Model of the episternal region of a mouse embryo (8·5 mm.). It shows a median nodule of procartilage, in front of the presternum, and between the sternal cornua. It was embedded in a mass of dense mesoderm, which forms a projecting keel for the attachment of the pectoral muscles.

septum (not represented in the model), which affords attachment on each side to fibres of the pectoralis major. The central cartilaginous nodule probably represents a rudiment of the interclavicle.

Caudal to the level of the inner ends of the clavicles and the central nodule are the cranial extremities of the two sternal bars, each of which is so bent laterally as to form a transverse bar which occupies the space between the clavicle and the sternal end of the first rib. If the sternal bars are traced in a caudal direction they are seen to course almost parallel with each other and to lie medial to the ventral extremities of the ribs. They are, however, separated from the ribs by a perichondrial layer which is common to both ribs and

sternum and is continuous with the perichondrial sheath of both rib and sternal bars.

The model shows a constriction of each sternal bar about midway between the first and second ribs. There is, however, no definite interruption of the sternal bars at these points. Constrictions are also present at the level of the lower borders of the second ribs and near the centres of the sternal ends of the third ribs. In the sections the mesodermal sternal bars are seen to enclose curved plates of procartilage, each of which has a ventral and a dorsal border, a convex medial surface and a concave lateral surface. The ventral borders

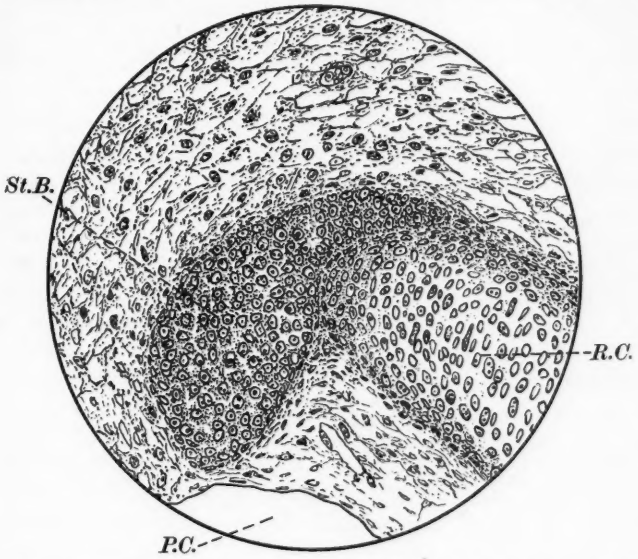

Fig. 23. Transverse section through a mouse embryo (8·5 mm.), showing the junction of a rib cartilage with the procartilage which is forming in the sternal bar. The matrix of the central part of the rib cartilage has already become quite clear, whereas the matrix of the sternal cartilage is still granular. The line of union of the rib cartilage with the sternal cartilage is also indicated by the arrangement of the cell elements.

afford attachment to the fibres of the pectoral muscles and the lateral surface is in relation with either the muscle of an intercostal space or the sternal end of a rib.

The constrictions probably indicate a secondary segmentation or division into hemisternebrae. The inner ends of the ribs are enlarged and are slightly bifurcated. In one or two sections through the articulation of each rib with the sternal bar, the perichondrium intervening between the tips of the ribs and sternum has disappeared and the rib appears to be continuous with the sternal bar, but a close inspection of the junction shows a definite concentric arrangement of the nuclei which are embedded in a clear matrix at the tip of

the rib (fig. 23). This is quite different from the arrangement of the cells of the procartilage of the sternal bar in which the nuclei are polygonal in shape and lie in a more granular matrix. If the sternal bars are followed backwards in the sections, they are found to diverge from one another and gradually disappear just beyond the tips of the seventh cartilages.

Model of the sternum, episternal region and clavicles of a rabbit embryo by Edward McLellan, F.R.C.S.

The embryo of 18 days' gestation measured 12·5 mm. in its greatest length. The sections were cut transversely and were 10 mm. in thickness. The part modelled, consisting of 816 sections was, therefore, about 8 mm. in length.

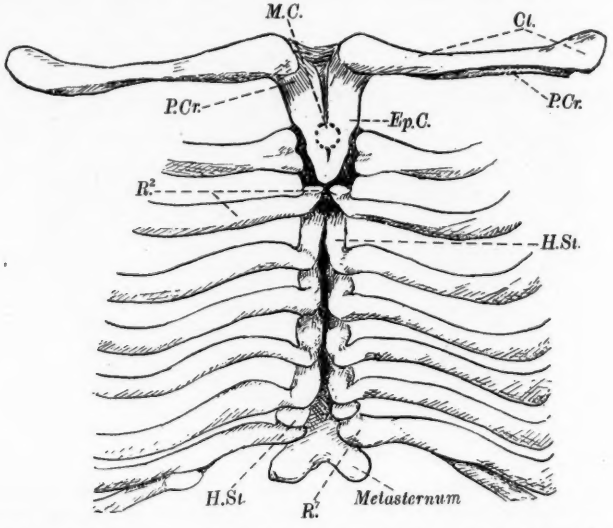

Fig. 24. Model of the sternum and ribs of an 18-day rabbit embryo, showing a median nodule of procartilage, in front of the sternal cornua. It was imbedded in a mass of condensed mesoderm, which formed a keel-like projection. The latter gives origin on either side of the pectoral muscles.

Every second section was drawn at a magnification of 50 diameters, and the drawings were rolled on wax plates 1 mm. in thickness. As was done in the model of the sternum of a mouse embryo, the oesophagus and the lumen of the trachea were utilised in determining an axial dorsal line and a midpoint was taken in front in order to provide a median ventral line.

The embryo shows a later stage in the chondrification of the sternum than that of the 8·5 mm. mouse embryo, the cartilaginous hemisternebrae are at this stage continuous with the cartilage of the ventral ends of the ribs and the histological structure of the two elements is indistinguishable. A slight

indication of the junction of the two structures is, however, distinctly visible in some of the sections. Right and left hemisternebrae have also united with each other in the median plane, but there is a distinct gap between the hemisternebrae of one segment and that of the segment in front or behind it.

The model (fig. 24) shows a general arrangement of the parts which is similar to that found in the model of the sternum of the mouse embryo, but in the later stage in the rabbit, the hemisternebrae have united in the median plane and the sternal cornua have also joined (figs. 25 A–F). The central core of the clavicle is now completely ossified; it is enveloped in a thick layer of dense mesoderm which is directly continuous with that of the sternal cornu, no indication of a sterno-clavicular joint having yet appeared.

There is a central core of cartilage in each sternal cornu and a layer of loose mesenchyme containing blood vessels between the cranial end of the sternum and the cornu on each side. The sternal cornua are well seen in fig. 25 A (slide 33). Between the medial ends of the clavicles and the first ribs on each side is a thickened mass of mesoderm a transverse section of which is cordate in outline, the notched border being dorsal and the apex directed ventrally (fig. 25 B). If this dense mesoderm is traced backwards, cartilage appears in its dorsal area on each side and a more ventrally disposed single median nodule of cartilage is found to be present. This nodule corresponds to the median nodule described in the mouse embryo and probably represents the interclavicle.

(The formation of cartilage in the development of a bone, such as the interclavicle, which is primarily membranous in origin, is no sure criterion for considering that because cartilage has appeared this cartilage must represent some structure other than membrane bone, for cartilage appears in the developmental history of both the mandible and the clavicle. In the former the skeletal element is primarily cartilaginous and in the latter the mesenchymal ossification must be regarded as primary.)

The Pectoral muscles arise from the keel-like projection on the ventral aspect of the thickened mesoderm. The right and left cartilaginous bars in the dorsal part of the dense mesoderm unite in the median plane opposite the articulations of the first ribs with the sternum, an indication of the junction being visible as a band of densely aggregated nuclei (fig. 25 C).

The articulations of the cartilages of the first pair of ribs with the sternal bars are characterised throughout by an intervening stratum of mesodermal cells continuous with the investing perichondrium of the sternum and ribs; the continuity of the cartilage of ribs and sternum, present at a later stage of development, has not yet been established.

Behind the first ribs and opposite the intercostal spaces between the first and second ribs the right and left hemisternebrae are completely fused, all indication of the bilateral origin of the sternal plates having disappeared.

Where the inner ends of the second ribs articulate with the sternum, there is a stratum of intervening perichondrium similar to that which is present in

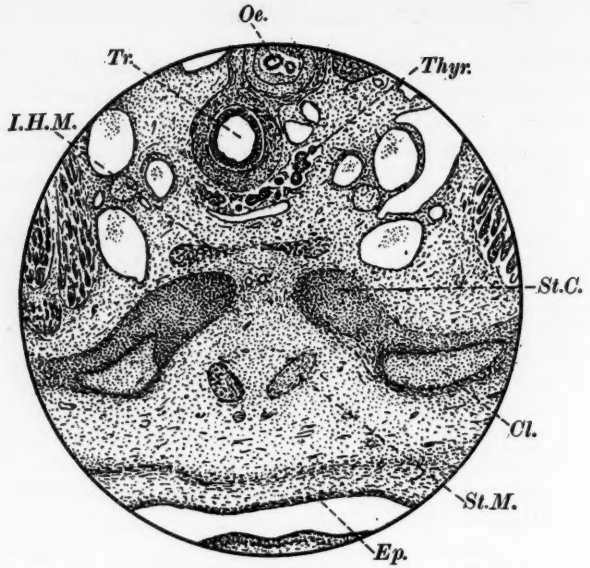

Fig. 25 A. Transverse section through an embryo rabbit (18 mm.), showing the cartilaginous inner ends of the clavicles. The perichondrium of the clavicle on each side is continuous with a bar of condensed mesoderm, the sternal cornu or rudiment of the omosternum. At a lower level this bar contains a central core of cartilage.

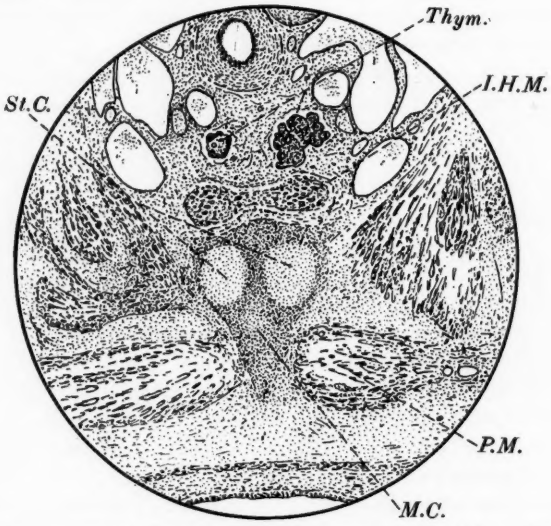

Fig. 25 B. Transverse section through the same embryo, passing through the region of the sternal cornua posterior to the clavicles. The right and left cartilages of the sternal cornua are cut across in front of the point where they join to form the cartilage of the presternum. Ventral to these, in the median plane, is a small nodule of procartilage. The condensed mesoderm which encloses these cartilages appears triangular in transverse section, the apex of the triangle being directed ventrally.

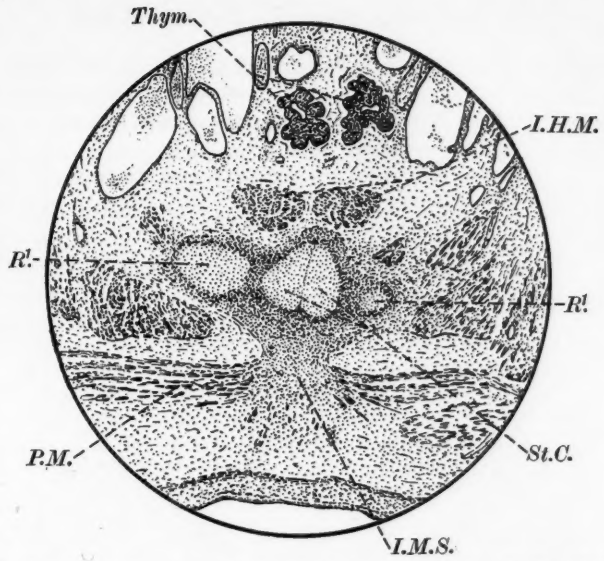

Fig. 25 C. Section through the same embryo, at the level of the ventral ends of the first ribs. These are separated from the sternal cartilage by a thick layer of perichondrium. The presternal cornua have fused in the median plane; they terminate below in a rounded point at the level of first intercostal space. Compare fig. 24.

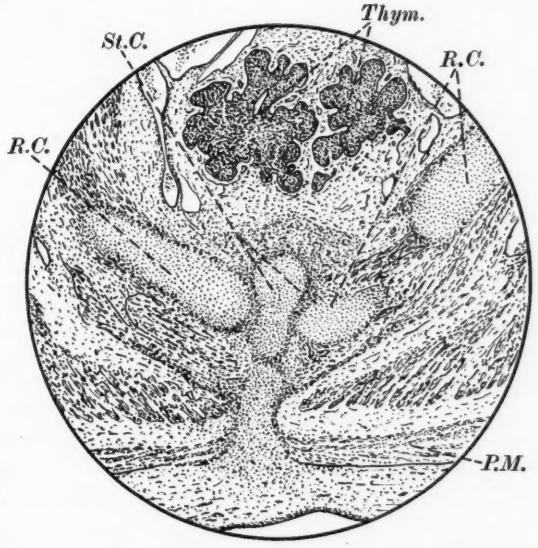

Fig. 25 D. Section through the same embryo showing the fusion of the sternal cartilages with each other in the median plane and the relation of these to the succeeding pair of sternal cartilages and to the rib cartilages.

the articulation of the first ribs with the sternum, but on reaching the level of the third ribs a direct continuity of rib cartilage with sternal cartilage is evident and also indications of a cleft between the right and left halves (hemisternebrae) of the sternal cartilage (figs. 25D and E).

On reaching the space between the sixth and seventh costal cartilages, the hemisternebrae are separated by some loose mesenchymal tissue. The connection between the costal cartilage and the hemisternebra on each side is similar to the relations of these at a higher level. At the level of the eighth

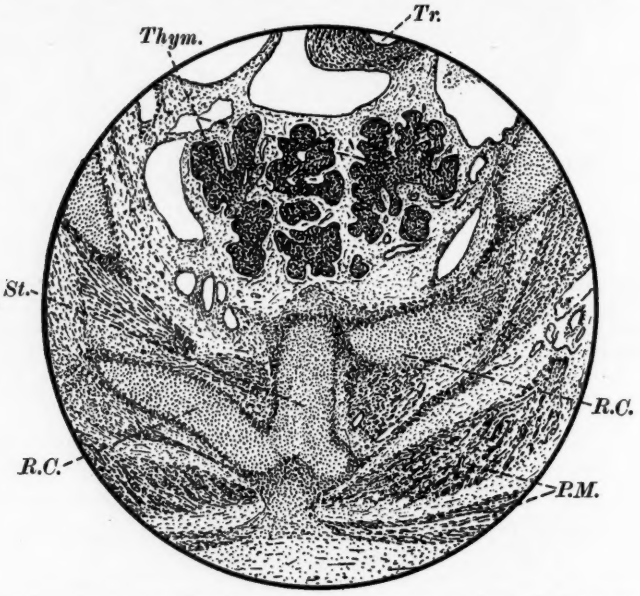

Fig. 25E. Transverse section through the same embryo showing continuity of the rib cartilage with the sternal cartilage established. The right and left hemisternebrae have also fused with each other in the median plane and the cartilaginous tissue of the two sides has become continuous. A slight constriction at the junction of the rib-cartilage with the cartilage of the hemisternebra marks their line of union, and a pale line in the centre where the nuclei of the cartilage are less numerous indicates the junction of the hemisternebrae with each other.

rib cartilage, the tip of the cartilage is completely separated by a wide interval from the hemisternebra which is continued backwards as a bifurcated xiphisternum in the region of the linea alba, in which it ends on each side as a blunt rounded process (fig. 25F).

There is no indication of any previous connection of the xiphisternum with the tips of either the eighth or ninth cartilages.

The appearances seen in the *late* cartilaginous stages of the developing sternum in rodents at the stage when the rib and sternal cartilages have become continuous would be extremely difficult to interpret, even with the help of

models, were it not for the conditions which are seen in the mesodermal and procartilaginous phases of these and other animals in which the independent chondrification of the mesodermal bars is more easily observed. Both the microscopical appearances and the model of the 8·5 mm. mouse embryo show this earlier procartilaginous stage in which there is a clear cut line separating hemisternebrae from costal cartilages. In later stages of mouse embryos and in the 12·5 mm. rabbit embryo, the costal cartilages have fused with the now fully differentiated cartilage of the sternal bars to such an extent that costal cartilages and sternal bars appear to have been primarily continuous; it is not surprising, therefore, that they have been considered so to be. Moreover, in

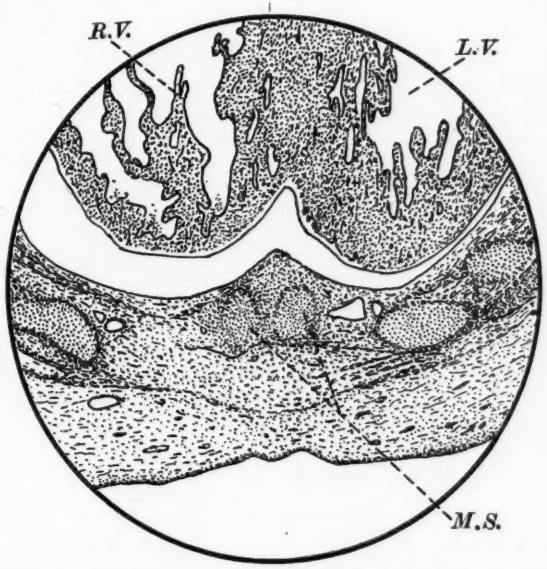

Fig. 25 F. Transverse section through the same embryo showing the prolongation of the sternal cartilages beyond the attachment of the last sternal ribs.

certain reptiles, Crocodilia, the Monotremes, and certain placental Mammals, e.g. the Cape Hyrax (*Procavia capensis*), the ventral ends of a number of ribs join together to form a pair of diverging bars which course backwards and laterally in the abdominal wall. These bars may be distinct from or blended with the metasternal cornua. It is the latter condition especially which suggests that the mesosternum and presternum may be formed in a similar manner by the fusion of the ventral ends of the more cranial ribs. The ribs may contribute to the formation of the sternum by growing into the mesodermal bars but the sternum arises primarily from the sternal bars and any participation of the ribs which may occur in its formation is secondary.

Development of the sternum in Man

In the human subject the mesodermal sternal bars (fig. 26) are formed essentially in the same way and have the same relations as in other Mammals. When they first appear, they lie in the axillary region, where they are closely associated with the origin of the pectoral muscles (fig. 16). They are joined secondarily by the ventral ends of the sternal ribs which at first consist of rod-like condensations of the fibrillar mesoderm constituting the myosepta. As the ribs elongate, the sternal bars move with them towards the median plane. Their union cranially is preceded and accompanied by a condensation

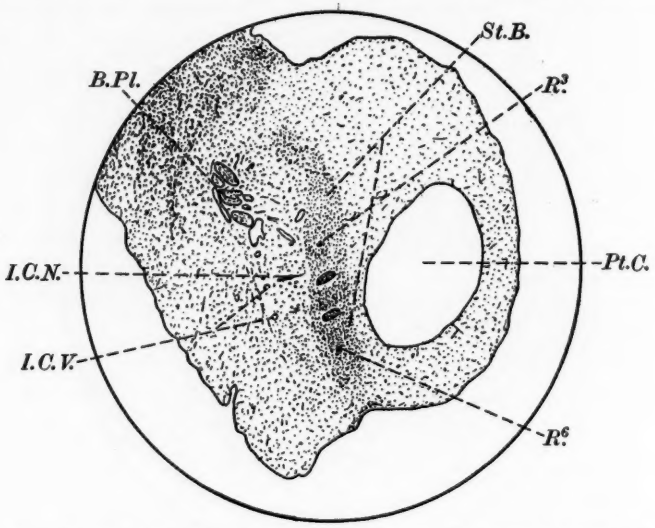

Fig. 26. Sagittal section of human embryo (12 mm.), showing the relation of the tips of the ribs to the mesodermal sternal bar. The section is near the axilla and shows a cross section through the brachial plexus and axillary vessels. The ribs are the third to the sixth inclusive.

of the mesenchyme between the clavicles and a growth of a median thickening of the mesenchyme in a caudal direction. This is plainly visible in embryos from 16 to 22 mm. in length, but not earlier (fig. 27). At a later stage, when the cartilaginous sternal bars have joined, there is a considerable thickening of the perichondrial layer in front of the cartilage. As the cartilage increases in thickness it does so at the expense of this layer. In other words, the perichondrial layer becomes thinner by the gradual conversion of its deeper layers into cartilage. The early median condensation of the mesoderm is very similar to that which occurs in Rodents and possibly represents a vestige of the interclavicle. There are also two lateral condensations of mesoderm in relation with the inner ends of the clavicles. These resemble the anterior sternal cornua of

the Rodent's sternum but are not nearly so conspicuous. They give rise to the ligamentous structures of the sternoclavicular joints including the interclavicular ligament and the interarticular fibrocartilage, and also, when they are present, to the episternal cartilages and the episternal bones which succeed the cartilages (figs. 19, 20).

In the human subject, the procartilaginous stage of development is, as in Rodents, difficult to observe. The mesodermal sternal bars and costal cartilages are at this period very closely associated. Moreover, the ribs being near together and their sternal ends turned upward, the exact process of chondrification is extremely difficult to determine. At the 22 mm. stage, however

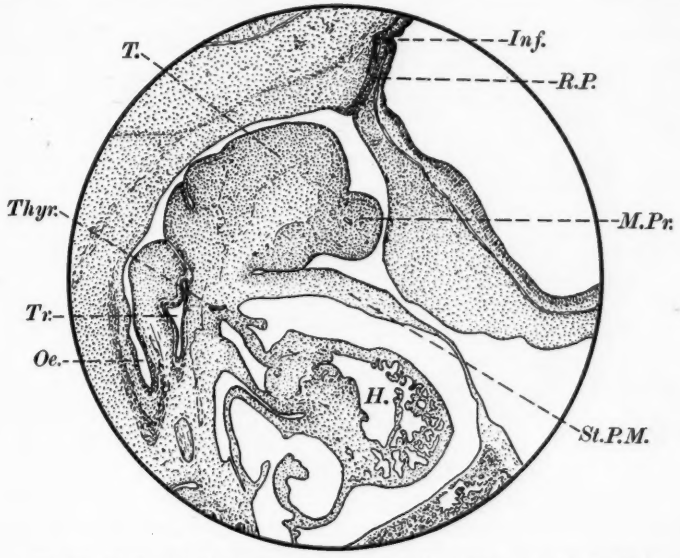

Fig. 27. Median sagittal section of a human embryo (12 mm.), showing the absence of any median condensation of the mesoderm at this stage.

(fig. 21), oval areas of procartilage in the mesoderm between the tips of the costal cartilages are distinctly visible. These, becoming cartilaginous, fuse with each other and with the costal cartilages before the sternal bars have completely united with each other in the median plane. The whole process of chondrification is extremely rapid and, when completed, little or no trace of the union of costal cartilages with sternal cartilage is left (figs. 18, 29). The mesodermal sternal bars are now replaced by two continuous longitudinal cartilaginous bars which join from before backwards and form a continuous median plate. When the two cartilaginous bars have completely joined their line of union disappears and it is only occasionally that evidence of the bilateral origin of the sternum is present after fusion has taken place. There is, more-

over, a continuity of the presternum with the mesosternum (fig. 18), and of the mesosternum with the metasternum. At a later stage definite syndesmoses and arthrodial joints between the costal cartilages and the sternum and between the different segments of the bony sternum make their appearance.

The later stages of ossification of the sternum and the variations of this process are so well known that we do not propose to deal with the period of development here. We should like, however, to draw attention to the inference made by Hanson with regard to the position of the ossific centres opposite

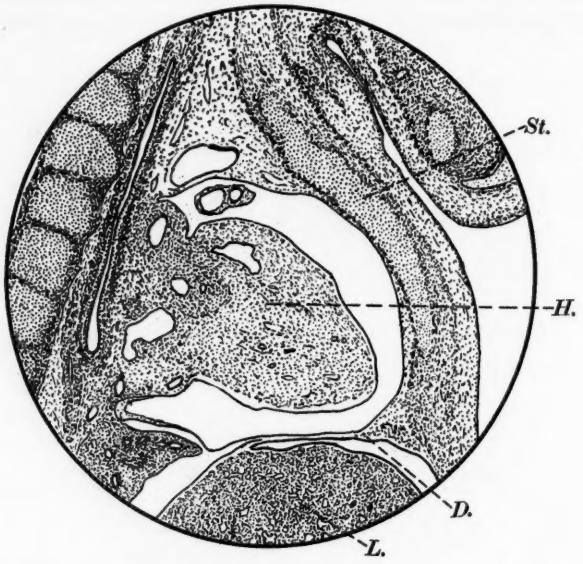

Fig. 28. Median section of a guinea-pig embryo, showing the sternum as a continuous cartilaginous plate. The upper parts of the two cartilaginous sternal bars have fused in the median plane, and all trace of their formation by the fusion of separate centres of chondrification has disappeared. The mesodermal bars are not yet completely chondrified. This is indicated by a condensation of the mesenchyme which extends beyond the cartilage into the abdominal wall below the level of the diaphragm, and also forms a thick sheath-like covering on the ventral aspect of the sternum. The thin upper part of the cartilaginous plate lies cranial to the junction of the first ribs with the sternum.

the intercostal space. He contends that if the sternum was costal in origin the ossific centres would be opposite the ribs, and that their development opposite the spaces is evidence in favour of a non-costal origin of the sternum.

It is not always safe to draw conclusions from congenital abnormalities or teratological material with regard to processes of normal development. Attention may be drawn, however, to the fact that, in certain cases of congenital defects of the ribs in the human subject in which the ventral ends of some of the sternal ribs including their costal cartilages have been absent,

the corresponding part of the sternum has been normally developed and is not deficient as surely would have been the case if the sternum were normally derived from the costal cartilages. A good example of such a case was one described by Benians.

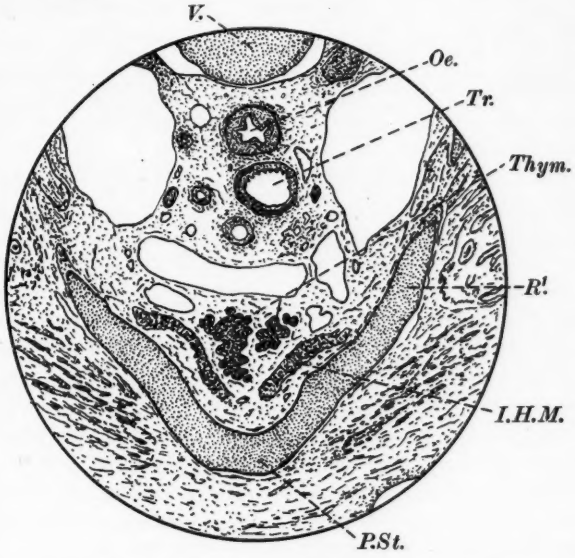

Fig. 29. Transverse section of a human embryo (41 mm.), showing the continuity of the costal cartilages of the first ribs with the sternum.

SUMMARY

1. The true sternum originates, in all classes of vertebrate animals, from two mesodermal plates or sternal bars.

2. The sternal bars are at first situated in the lateral part of the body wall, posterior to the coracoids, with which in some types of animals they are primarily continuous.

3. The mesodermal sternal bars subsequently become chondrified and, having moved towards the mid-ventral line, fuse with each other from before backwards and thus form a median cartilaginous plate.

4. A median bar and two lateral nodules of condensed mesoderm appear in the "pro-" or "episternal" region of some Mammals.

5. The mesodermal sternal bars and "episternal" structures originate independently of the ribs.

6. The mode of chondrification of the mesodermal sternal bars varies in different classes of animals. A gradual transformation may take place of the mesodermal plate or bar into cartilage, a procartilaginous stage intervening. This change commences in the centre, spreads to the margins of the plate and

into secondary processes. In some forms separate centres of chondrification appear at the lateral borders of the plates and between the ends of the ribs bounding an intercostal space. The separate centres of chondrification fuse with one another to form a uniform cartilaginous plate which loses, for a period, its earlier segmented appearance. This, however, reappears in some types with the formation of ossific centres opposite the intercostal spaces.

7. Segmentation of the sternum occurs in some Mammals and Reptiles. It is a secondary condition which affords a certain amount of mobility and resilience to the thorax.

8. The true metasternum is formed as a backward prolongation of the sternal bars.

9. The epicoracoid cartilages and the interclavicle may participate in the formation of the presternum. The extent to which they enter into its formation diminishes in ascending from lower to higher types of vertebrate animals.

10. The sternal segments of the last two cervical and the four thoracic ribs of *Gallus domesticus* appear as separate centres of procartilage, in contact with the lateral borders of the mesodermal sternal plates. The first two centres, which correspond to the sternal segments of the last two cervical ribs, degenerate during embryonic life; consequently these two costal arches are not completed and the corresponding vertebral segments end in free extremities. The four succeeding procartilaginous centres develop laterally in the myosepta and form the sternal segments of the corresponding ribs. They take no part in the formation of the sternum.

11. The sternal cornua of cursorial birds represent the sternal segments of the last pair of cervical ribs and are most probably developed from independent centres of chondrification appearing in the ventral ends of the corresponding myosepta.

12. The sternal cornua which are sometimes present in cases of cervical rib and rudimentary first thoracic rib in the human subject are similar in nature to the costal cornua of cursorial birds and like these are homologous with the sternal segment of the corresponding rib. The fibrous cord which joins the sternal cornu to the tip of the vertebral segment of the rib represents an unossified portion of the myoseptum.

13. The irregularities in articulation of the ribs with the sternum which are met with in anomalous cases of suppression or supernumerary ribs are readily explained on the basis of the articulation of the ribs with the sternum being secondary. The independent origin of the sternum is further confirmed by the development of the corresponding half of the bone in cases of suppression of several ribs on one side (Benians).

14. There is a definite correlation between the length of the sternum and the number of ribs which articulate with it (Anthony, Tredgold). This is, however, an adaptive relation similar to the increase in size of the carina, *pari passu*, with the increase in size of the pectoral muscles; it does not imply

that the added or reduced parts of the sternum have originated or failed to originate from the ribs concerned.

In conclusion, we wish to express our thanks to Prof. E. Barclay-Smith for criticism and for the kind interest which he has taken in revising the manuscript.

We also wish to thank Mr J. Weston who has prepared most of the serial sections which we have made use of, and rendered valuable assistance in the construction of the models.

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EXPLANATION OF ABBREVIATIONS

Ad.T.	Auditory tube.
Ao.	Aorta.
B.Pl.	Brachial plexus.
C.C.	Coracoid cartilage.
C.Ch.	Centre of chondrification.
C.R.	Cervical rib.
C.R.′	Cervical rib.
C.R.″	Cervical rib.
C.V.	Cervical vertebra.
Cl.	Clavicle.
Clm.	Cleithrum.
Co.	Coracoid.
Co.C.	Condylar cartilage.
Co.Sc.	Coraco-scapular cartilage.
D.	Diaphragm.
E.Pt.	External pterygoid muscle.
E.St.C.	Episternal cartilage.
Ect.Ost.	Ectosteal bone.
Ep.	Epidermis.
Ep.C.	Epicoracoid cartilage.
F.	Fenestra.
F.C.	Fibro-cartilage.
F.E.C.	Epicondylar foramen.
F.L.	Fibrous layer.
F.Sc.	Scapular foramen.
G.G.	Genital gland.
Gl.	Glenoid cavity.
H.	Humerus.
H.St.	Hemisternebra.
Ht.	Heart.
I.A.P.	Interarticular plate.
I.Cl.	Interclavicle (episternum).
I.C.L.	Interclavicular ligament.
I.C.N.	Intercostal nerve.
I.C.V.	Intercostal vessels.
I.H.M.	Infrahyoid muscles.
I.M.A.	Internal mammary artery.
I.M.S.	Intermuscular septum.
I.Pt.	Internal pterygoid muscle.
Inf.	Infundibulum.
L.	Liver.
L.A.	Left atrium.
L.V.	Left ventricle.
M.	Masseter muscle.
M.B.	Membrane bone.
M.C.	Median nodule of procartilage.
M.N.	Mesonephros.
M.Pr.	Mandibular process.
M.S.	Metasternum.
M.St.B.	Mesodermal sternal bar.
Mk.C.	Meckel's cartilage.
N.Ch.	Notochord.
Oe.	Oesophagus.
Op.C.	Opercular cavity.
Op.F.	Opercular fold.
Ot.G.	Otic ganglion.
P.	Pericardium.
P.B.	Periosteal bone.
P.C.	Pericardial cavity.
P.Cr.	Procoracoid.
P.D.	Parotid duct.
P.M.	Pectoral muscle.
P.N.	Precoracoid notch.
P.St.	Presternum.
Pl.O.	Pleurosteon.
Pr.C.C.	Procoracoid cartilage.
Pt.C.	Peritoneal cavity.
R.	Rib.
R.A.	Right atrium.
R.Abd.	Rectus abdominis.
R.C.	Rib cartilage.
R.P.	Rathke's pouch.
R.V.	Right ventricle.
S.C.	Sternal cartilage.
Sc.	Scapula.
S.C.M.	Sterno-coracoid membrane.
S.G.	Submaxillary gland.
Sq.	Squamosal.
S.Sc.C.	Suprascapular cartilage.
St.	Sternum.
St.B.	Sternal bar.
St.C.	Sternal cornu.
St.M.	Tendon of sterno-mastoid.
St.P.M.	Sterno-pericardial membrane.
St.R.	Sternal rib.
St.R.^{1}	Sternal rib
St.R.^{2}	Sternal rib.
St.R.^{3}	Sternal rib.
T.	Tongue.
T.I.	Tendinous intersection.
T.V.P.	Tensor veli palatini.
Th.R.	Thoracic rib.
Thym.	Thymus gland.
Thyr.	Thyroid gland.
Tr.	Trachea.
V.	Vertebra.

STUDIES IN ACHONDROPLASIA

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I. DYSTROPHIA HORMATHICA[1], AN UNRECORDED FORM OF DEFECTIVE OSSIFICATION AKIN TO ACHONDROPLASIA

This specimen is unique; no reference to any similar case has been found. It is a male foetus of eight months, well covered, with short flipper-like extremities[2]. Rokitansky described a foetus with very short flipper-like extremities and a peculiarly flat face, and to this he gave the name "Phocomelus." In Ballantyne's *Antenatal Pathology* a photograph of another specimen of phocomelus is given, and in 1927 Harris wrote of a specimen which came into his hands. While the external features of these foetuses resemble most closely the specimen here described, the skeleton is entirely different.

In the skull, the vault is normally developed; in the base, the great wing of the sphenoid, its pterygoid processes, the petrous temporal and tympanic ring, and the ex- and post-occipital bones, are developed normally. The basi-occiput—basi-sphenoid alone shows evidence of any abnormality; its occipital ossific centre is short, narrow and separated from the sphenoid centre by a hypertrophied mass of cartilage which preserves the form of the dorsum sellae but is grossly enlarged.

The maxilla is considerably rotated on a transverse axis, the alveolus being very retracted; the hard palate is foreshortened, is normal in width, looking backwards and downwards. Otherwise the facial skeleton is well formed. The teeth, unerupted, are developed normally.

The vertebral bodies consist of masses of cartilage, twice the usual size, with exceedingly small ossific centres in the lumbar region and none elsewhere. The cervical neural arches are well ossified, the dorsal and lumbar fairly well, the latter lagging a little.

In the pelvis, the ilium and ischium possess ossific centres much retarded in extent; no pubic centre is present, and the cartilage of the whole pelvis is considerably over-developed.

The clavicles are perfectly natural and well formed. The ribs are well developed in bone from the heads to the angles—in front of this they consist of swollen cartilage.

[1] ὁρμαθός = chain of beads. This name was suggested by Mr W. R. Le Fanu, Librarian to the Royal College of Surgeons, England.

[2] The author is indebted to Mr C. P. G. Wakeley for placing this specimen at his disposal. The specimen is preserved in the Museum of the Royal College of Surgeons, England.

Fig. 1A.

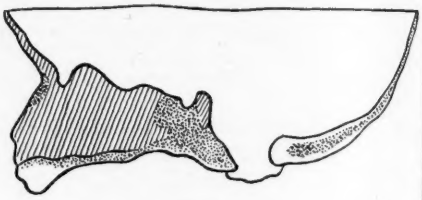


Fig. 1B.

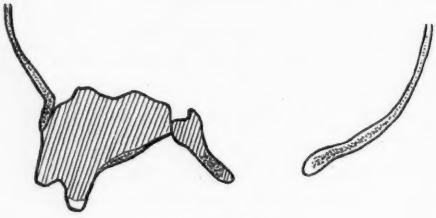


Fig. 1C.

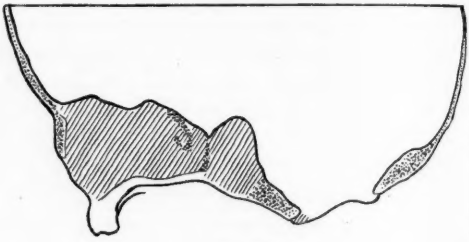


Fig. 1D.

Fig. 1. Median sagittal sections of ninth month foetal skulls to illustrate comparative growth changes. A, normal; B, achondroplast; C, variation in achondroplasia (see section III); D, foetus of section I.

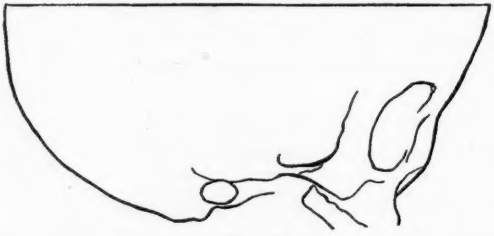


Fig. 2A.

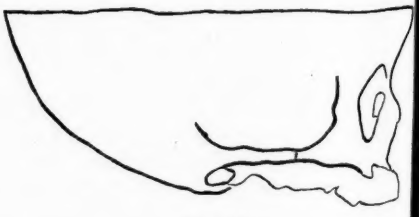


Fig. 2B.

Fig. 2. Lateral view of facial skeleton of skulls: A, foetus of section I; B, achondroplast foetus.

The long bones of the extremities present the outstanding features of the disease. In all of them the diaphyseal shafts are scarcely a quarter of an inch in length, yet in width equal to those of a normal foetus of the same age, formed of bone, and expanded at each end. All the epiphyses are huge spherical masses of cartilage, resembling the normal in gross form alone.

The elements of the tarsus and carpus are entirely cartilaginous, except the terminal phalanges which possess ossific centres. The excess development of the cartilage is less marked in the hands and feet; and there is a complete failure in the metatarsal osseous development.

MICROSCOPIC ANATOMY

In the epiphyses the apparent inactivity of the cartilage is the striking feature. The cells are scanty in the field; the intercellular matrix is exceedingly attenuated and poorly staining; and there is a complete absence of the fusiform and active cartilage cell. A typical cell is about 7 μ, rounded, cytoplasm poorly stained, nucleus rounded, well stained with four or five deeply staining nucleoli. The space surrounding the cell is very indefinitely marked.

As the epiphyseal line is approached the cells scarcely increase in size and there is nothing even approaching the vigour and activity of the normal growth line; there is very little evidence of any division of cartilage cells. Somewhat enlarged hydropic cartilage cells may be seen lying quite inactively among the osteogenic mesenchyme. There is no preliminary calcification in cartilage and no "preparatory scaffolding." The osteogenic mesenchyme is slowly pushing its way into the very attenuated matrix and among the cartilage cells.

The bone-forming tissue is quite active, spicules of bone being formed right up to the cartilage—the texture of the bone is not as fine as is normally the case.

The vertebral bodies consist of an extraordinary cartilage—very large rounded swollen structureless cells appear to have undergone a hyaline degeneration; the nuclei are small irregular pyknotic and degenerate structures. There is a scanty fibrillar intercellular matrix, quite unlike the normal homogeneous matrix. From the very small centre of ossification which is present bone growth is spreading in a slow fibrillar fashion.

THE DUCTLESS GLANDS

The thyroid gland was larger than usual, and the same remark applies to all the glands. The alveoli consist of solid masses of very plentiful cells, rounded, small and staining well. No vesicular lumina are present, and no colloid. The interalveolar stroma is delicate, contains no lymphocytes, and is very richly supplied with capillaries.

The parathyroid glands could not be discovered with the naked eye or in serial section; many likely-looking nodules proved to be thymic or lymph-glandular.

The anterior lobe of the pituitary is somewhat larger than normal, is very vascular, and its general microscopic structure closely resembles the normal; the pars intermedia is well represented by large irregular alveoli with a double layer of cells surrounding a larger lumen. The posterior lobe is a very small area almost completely surrounded by the pars intermedia and rather scantily supplied with cells.

DISCUSSION

The outstanding feature in this skeletal disease is the curious incidence of the bony affliction. Alongside the merest caricatures of bones are normally developed skeletal parts; in an almost normal skull is a cartilaginous dystrophy of the basi-occiput; in the shoulder girdle the clavicle is normal, the scapula a swollen misshapen cartilaginous mass. The neural arches of the vertebrae are moderately well formed and ossified; the bodies are large cartilaginous masses with scarcely the merest rudiment of ossification.

Is there any explanation of such an incidence of the disease? Is one form of ossification affected while another escapes? There is not merely a failure of ossification at the epiphyseal line as in achondroplasia. The freedom from disease of the skull vault, the facial skeleton, and the clavicle would in itself suggest that "membrane" ossification was unaffected, while perhaps the disease was one involving ossification in cartilage. It does not appear to be sufficient to make the assumption that "membrane" or "dermal" ossification escapes the abnormal growth process, but if we extend the meaning of the term "membrane" and use in its place "sub-periosteal" ossification, it will then appear that nowhere in this disease is sub-periosteally formed bone maldeveloped. In support of this contention, the normal skull vault, facial skeleton and clavicle, and the normal *diameter* of the shafts of the long bones may be cited.

But when we enquire further whether the disease is one affecting ossification in cartilage, difficulties arise. Certainly every bone which is affected develops by ossification in cartilage—the basi-occiput, vertebral bodies, pelvis, the growth in length of the long bones and metatarsal bones. But there are certain bones which, primarily cartilaginous, have escaped the disease—the petrous temporal, the basal portion of the great wing of the sphenoid, the ex- and post-occipital. There seems no reason why some "cartilage" bones should escape, others suffer. Perhaps it is significant that all the normal "cartilage" bones lie in the base of the skull.

The histological findings reveal that the fault lies in the cartilage cells; they fail to develop in an orderly manner, do not respond to influences at the growth line, and assume a perverted activity. The influences causing the cartilage cells to behave in this fashion are unknown; but it seems certain that the failure of some ossific centres to appear, and the gross delay in others, is entirely dependent on the disordered cartilaginous growth.

Any hypothetical time factor or age incidence for the disease fails to afford

any explanation. The absence of any gross lesion in the ductless glands (with perhaps the exception of the parathyroids which were apparently unformed) and the peculiar incidence of the lesion suggest very strongly that we are dealing with a failure of one part of the mechanism by which cartilage is converted into bone at epiphyseal lines and around ossific centres.

II. A PECULIAR FORM OF ACHONDROPLASIA IN A PREDYNASTIC EGYPTIAN

This specimen is a skeleton of a dwarf[1] from Egypt—the earliest and most peculiar example of an achondroplasia-like disease known to us. For this skeleton the Royal College of Surgeons is indebted to Prof. Douglas Derry and Aly Bey Ibrahim. It was found by Mr Guy Brunton who assigns it to a very early period—the Badarian. The historic interest and the unique features of dwarfism make this skeleton worthy of close study.

Dioptographic drawings have been made of all the long bones, and these diagrams almost speak for themselves. They have been reproduced half natural size, and when this is appreciated the association of these bones with the skull presented (reduced to one third) will seem a very remarkable thing. So much so that the suggestion has been made that they are not part of the same skeleton. But another specimen is described in this paper which shows that even more remarkable discrepancies can occur in these bone diseases which begin in foetal periods. In the Dachshund the skull also is unaffected.

The skull appears to be quite free of any stigmata of skeletal disease. Tracings of a normal Dinka skull show a very close correspondence with the Badarian skull. There is no nasal retraction, no foreshortening of the cranial base, and no abnormality of the basicranial axis beyond a slight flattening of the angle of the base. So there are no indications that the growth failure, so marked in the limbs, has affected the skull in any way. The mandible has responded to the usual growth changes quite normally.

The tracings of the clavicles reveal that there is not very much deficiency in length, although the bones are very slender; there is certainly nothing approaching the gross discrepancy shown by the other long bones. As happens so often in growth diseases of the skeleton, the clavicles and the skull escape altogether.

The radii are shown as remarkably small bones, almost perfect miniatures of the normal, and they are bilaterally symmetrical. The set of the head is much more oblique than the normal; the radial tuberosity and the ligamentous prominences about the lower end are unusually pronounced.

The same remarks apply to the ulnae, again bilaterally symmetrical bones, perfectly in keeping with the radii. Unusually strong bony ridges mark the limits of attachment of the pronator quadratus.

The humeri show a departure from the symmetry which has been such a

[1] Preserved in the Museum of the Royal College of Surgeons, England.

Fig. 3. Skull of Badarian dwarf. Norma lateralis.

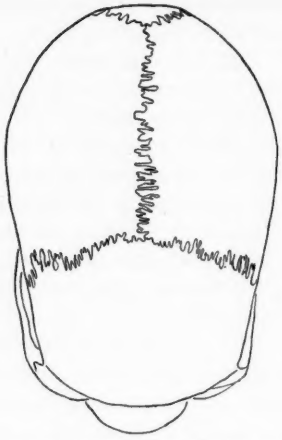


Fig. 4. Skull of Badarian dwarf.
Norma verticalis.

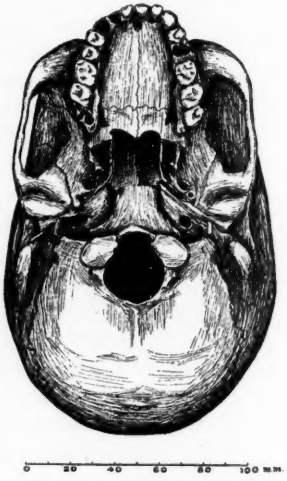


Fig. 5. Skull of Badarian dwarf.
Norma basalis.

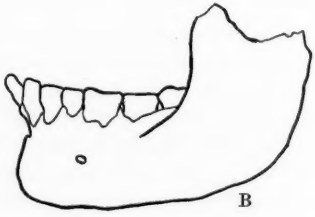


Fig. 6 A and B. Mandible of Badarian dwarf.

Fig. 7. Clavicles of Badarian dwarf: A, right clavicle from above; B, right clavicle—front view; C, left clavicle from above; D, left clavicle—front view.

Fig. 8. Badarian radii: A, left radius—anterior view; B, left radius—lateral view; C, right radius—anterior view; D, right radius—lateral view.

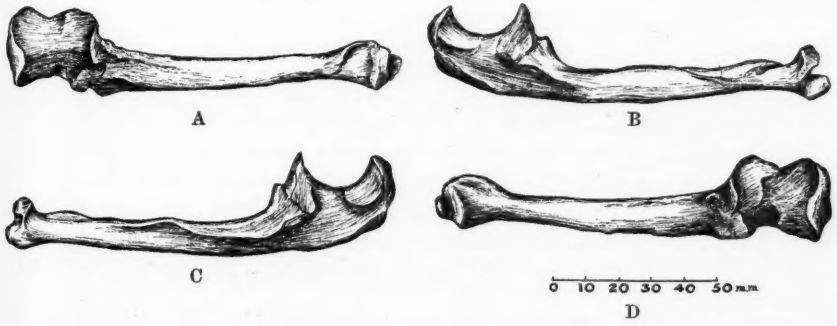


Fig. 9. Badarian ulnae: A and B, right ulna; C and D, left ulna.

marked feature in the bones just described. The right bone is of the same build as the other bones. The articular surfaces of the shoulder and elbow joints both appear to have undergone attrition, perhaps arthritic; the head is "set" at a low level as compared with the tuberosities. The muscular and ligamentous prominences are in keeping with the build of the bone.

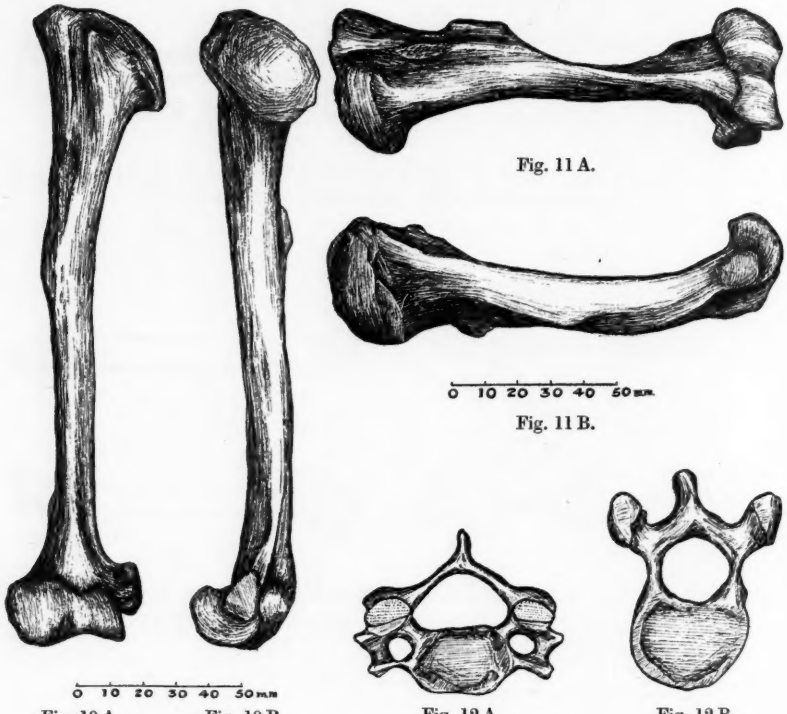


Fig. 11 A.

Fig. 11 B.

Fig. 10 A. Fig. 10 B. Fig. 12 A. Fig. 12 B.

Fig. 10. Badarian right humerus: A, anterior view; B, view from inner aspect.
Fig. 11. Badarian left humerus: A, anterior view; B, inner aspect.
Fig. 12. Badarian vertebrae: A, cervical; B, thoracic.

But, as can be seen from the drawings, the left humerus is built quite differently. It is much shorter; the muscular prominences are very stout, especially the deltoid tuberosity (this is even more marked than the figures would suggest); and the upper half of the bone is quite bulky in comparison with the lower portion. The head is grossly pitted about the fovea, perhaps due to maldevelopment, is "set low," and is very irregular, quite lacking the usual even contour.

The small bones of the hand and the ribs are quite in keeping with the other bones, as are also the scapulae. The glenoid articular surfaces show the

changes already seen in the humeral heads, a heaping up in some parts, attrition in others, a general convexity instead of a concave articulating surface.

X-ray photographs reveal very little departure from the normal in the texture of the bones.

It can be definitely said that this is not an example of achondroplasia as we usually know it. There is an absence of the typical skull changes, but that is not unknown in the disease; the limb-bones are short, but their diameter is also markedly reduced, not an achondroplast feature. Not only has the activity at the diaphyseal growth line been reduced, but subperiosteal activity has been brought to a correspondingly lower plane.

III. A VARIATION IN ACHONDROPLASIA

An eight months female foetus presented features familiar to French ob servers in this subject.

The limb bones were quite typically achondroplast, being short but well-developed otherwise; the epiphyses were normal for that age.

The unusual features were found in the basiocciput, vertebrae and pelvis. These bones were quite normal in form and size, but a very marked arrest of ossification has occurred. In the lower dorsal and upper lumbar vertebrae very small ossific centres are just visible; the other vertebrae and the neural arches possess no ossific centres. The basioccipital centre is present but is small, and most of the basisphenoid-basiocciput is cartilaginous. The ischium and the pubis have not commenced to ossify, and the iliac centre is inadequately developed for an eight months foetus.

The remainder of the cranial base and vault, and the facial skeleton are quite normal.

Thus in this skeleton the limbs are typical of achondroplasia, and the neurocentral axis shows such an inhibition of ossification as is unknown in that disease. When this foetus and that described in another part of this paper are compared, it seems that they form progressive stepping-stones away from achondroplasia, illustrating the gradation that occurs in foetal skeletal diseases and the admixture of dystrophies and specific disturbances that do occur. They also emphasize the association of the basicranial axis with the vertebrae in these skeletal diseases.

IV. THE NATURE OF ACHONDROPLASIA

The purpose of this study was to determine (1) if there was one fault in development which alone accounted for the skeletal errors of the adult, and (2) whether that fault was dependent upon some ductless gland disturbance.

The second phase of the inquiry was without definite result. Much of the material had been preserved, often faultily, for long periods. The only

Fig. 13.

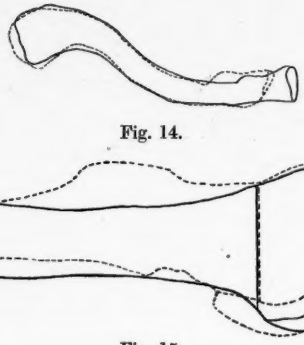


Fig. 14.

Fig. 15.

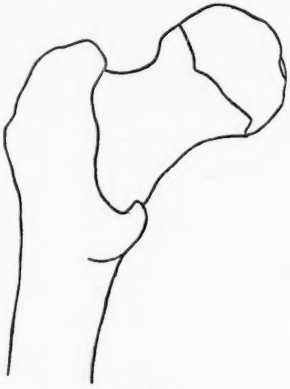


Fig. 16 A.

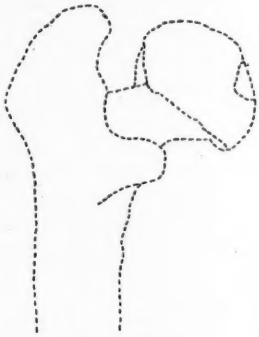


Fig. 16 B.

Fig. 13. Achondroplast femur (broken outline) superimposed upon normal femur, showing the equality in diameter.

Fig. 14. Achondroplast clavicle (broken outline) superimposed upon normal clavicle, demonstrating the approximate equality.

Fig. 15. Achondroplast humerus (broken outline) superimposed upon normal humerus, illustrating the normal volume of the epiphyses in achondroplasia and suggesting that the caput epiphysis slips at the anatomical neck at an early stage.

Fig. 16. A, normal; B, achondroplast—illustrating equality of epiphyseal volume.

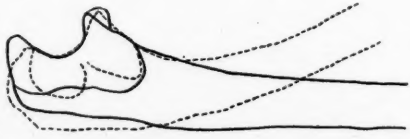


Fig. 17. Achondroplast ulna (broken outline) compared with normal ulna.

suggestive feature was a great increase in the eosinophile cells of the pituitary gland.

In the study of the developmental error, outline tracings were made, with the Leakey apparatus, of the bones of an adult achondroplast of African negroid origin, and of the normal bones of a similar African negroid. A few tracings are reproduced and almost speak for themselves. They show that the shafts of the long bones, while short, are in all other respects exactly equal to the normal—the muscular prominences are stronger, perhaps because of the great muscularity of these dwarfs.

The epiphyses are quite as well developed as the normal, and the tracings illustrate this rather strikingly. This is surprising, for up to a certain stage the ossification of an epiphysis occurs in cartilage; later its increase is from subperiosteal bone depositions—the first method of growth has not been at fault.

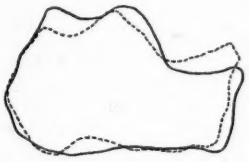


Fig. 18.

Fig. 19.

Fig. 18. Os calcis in achondroplasia (broken outline) superimposed upon the normal.

Fig. 19. Median sagittal section of skull in achondroplasia (broken outline) compared with section of normal skull. Also compare fig. 1 B.

Tracings of the os calcis and other tarsal and carpal bones reveal no deficiency in size or build. And this is quite significant for, up to quite an advanced stage in childhood, these bones are being formed by expansion of their cartilage and ossification is proceeding in cartilage.

Striking changes exist in the vertebrae and in the basicranial axis. As is well known, synostosis of the basiocciput and sphenoid occurs in these dwarfs some time before birth. This alone is responsible for the nasal retraction and increased area of the vault.

As an interesting side-light on the development of the base of the skull, it is instructive to compare the median sagittal sections of the foetal and the adult achondroplast skulls; the basicranial axis has scarcely altered a millimetre in length, and yet the foramen magnum has increased in size markedly, as have the ex- and post-occipital bones—features which show the active accommodative bone resorption and deposition which is occurring. That this resorption and deposition is quite normal in achondroplasia is illustrated by the tracings of the mandible which show that growth has occurred just as in the normal adult, with the normal accommodative changes for all the teeth.

The spinal canal is diminished in calibre, more so in some sections than in others; and the fault in the vertebrae corresponds to that of the basicranial axis, a premature synostosis, this time of the growth line between the centrum and the pedicles. The increase in the canal which occurs in the growing achondroplast is again one of accommodative bone resorption and deposition.

The bones "laid down in membrane," and the clavicles and ribs are of usual adult development.

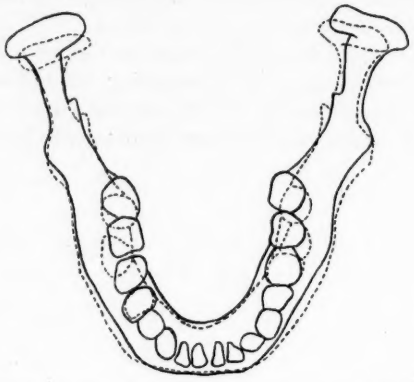


Fig. 20. Achondroplast mandible (broken outline) superimposed upon normal mandible.

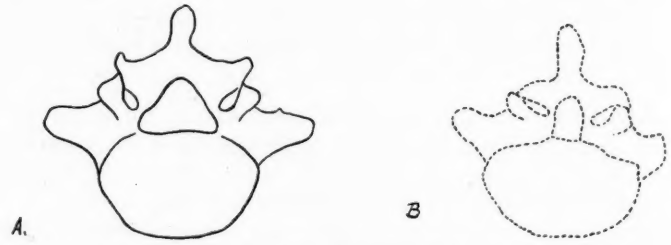
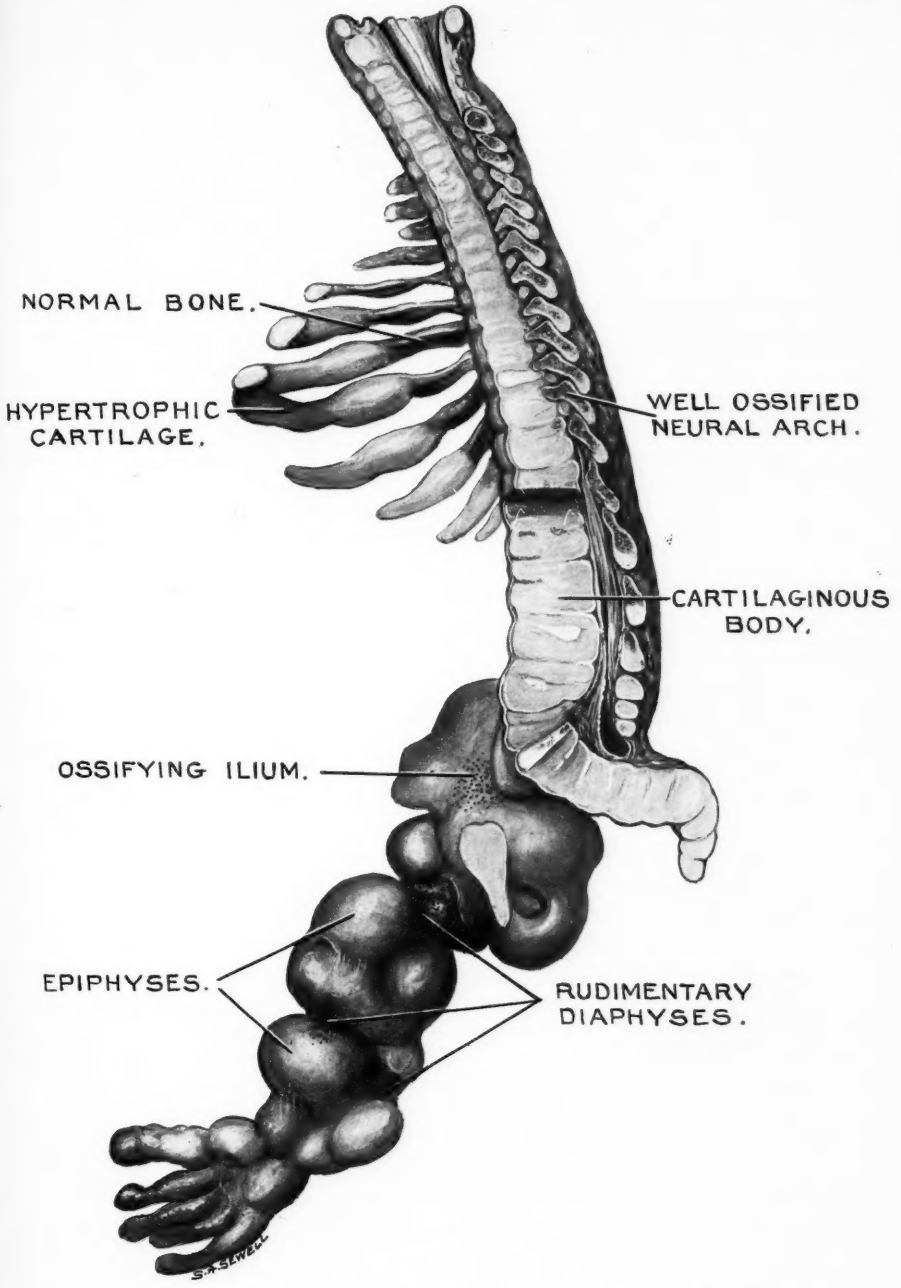


Fig. 21. Lumbar vertebrae: A, normal; B, achondroplasia.

Microscopic examination of the metatarsal bones of the achondroplast foetus shows the typical well-known lesion, the deficiency in the palisade arrangement of the cartilage cells at the growth line; the palisade formation does occur but it is very shallow, for example, three cells deep instead of twenty. This holds up the formation of a scaffolding for the osteoblasts; this scaffold is necessarily much shallower than is usually the case. The osteoblasts are normally active and deposit strong bone right up to the limits of the prepared area.

Some sections show a feature to which Lawford Knaggs has drawn attention—a fold of periosteum stretching some way across the growth line leaving



A drawing of the right half of the skeleton of the foetus described in the text.

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only the central part of this area free. But sections of other bones, especially the metatarsal growth lines, are quite free of this periosteal invasion. It appears that it is quite a secondary result and plays no part in the failure of growth—the growth in length is much below normal, the growth in diameter, on the other hand, is normal, and the periosteum is increasing *pari passu* with increase in diameter; the retardation at the growth line drags the periosteum in and so forms the "periosteal diaphragm."

CAUSE OF THE GROWTH FAILURE

The fault lies in the palisade proliferation of the cartilage cells. The ordinary proliferation of cartilage, as in the epiphyses and the tarsal bones, is normal.

In the very severe grades of achondroplasia with death *in utero*, quite a degree of growth in length of the shaft has occurred—the palisade proliferation is severely checked but not abolished. In those who survive, the retardation of growth occurs at an even rate throughout childhood and adolescence—achondroplasia is *not* a disease of one growth-period alone.

It is interesting to speculate why the cartilage cells should adopt the palisade arrangement. The cells have been actively proliferating in the epiphysis—why this special form of proliferation at the growth line?

A suggestion arises from the study of achondroplasia—that normally the cartilage cells respond to the presence of the osteoblasts by proliferating in a directional or polar fashion, and so prepare the scaffold for the osteoblasts to build upon. This "Cytoclesis" or "the call of cell to cell" is well recognized elsewhere—the epithelium of the head becomes transparent where it overlies the optic vesicle; and again, Speeman has shown the polar or directional force which an area of the blastopore exerts in determining the growth-changes of the embryo of the sea-urchin.

But at the growth line in achondroplasia this influence of the osteoblast on the cartilage cell is not sufficient—the cytoclesis is present, the osteoblasts and cartilage cells seem quite healthy and fully functional. So the suggestion arises that a catalyst is necessary to sensitize, as it were, the response of the cartilage cells. And the irresistibly easy thing to suggest is that a ductless gland product is the catalyst. And perhaps in achondroplasia this catalyst is insufficiently produced. Either this, or an excess of some ductless gland secretion actually inhibits the palisade proliferation at the growth line.

Careful microscopic examination of the glands in fresh material is still needed. And the tissue culture of whole chicken bone, as elaborated by Miss Fell, may form a new method of attack on the problem.

The features which need emphasis are the specificity of the incidence of the disease; the entirely normal bone formation in all other phases of bone growth; and the even continuity of the disease until the growth line is obliterated and adolescence passed.

THE LAWS OF MUSCLE AND TENDON GROWTH

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Woollard in his *Recent Advances in Anatomy* states that the preliminary phases of development are "succeeded by a final period of functional differentiation and co-ordination of the elements of the organism," and that "this correlation takes place through hormones, through the nervous system, by atrophy and hypertrophy, and by the mutual interaction of parts with one another (4)." Remarkably little is known of these processes. This paper is an attempt to unravel what appears to be the easiest group of problems of this period, that of the later development of muscles and their associated tendons. The attempt has been made along purely morphological lines, but it is hoped that other investigators will eventually confirm or disprove the conclusions by experimental research.

A problem is often most easily studied in its more extreme aspects, so in this case the plantaris, the muscle possessing the longest tendon in the human body, may serve as an introduction to our problem (fig. 1). In the normal adult figured the belly, measuring 110 mm., gives origin to a tendon 200 mm. in length. This discrepancy between flesh and sinew is still further emphasised by the separation of an individual macroscopic element of the belly (fig. 1, *P.*) which measures only 52 mm.

But when the same dissection is carried out on the gastrocnemius, the element so isolated (fig. 1, *G.*) measures precisely the same, 52 mm., though the gastrocnemius is a normally proportioned muscle. The apparent fleshiness is due to the greater distribution of the contractile tissue over the body of the muscle.

The identity in the lengths of the fibres taken from these two muscles suggests at once a common cause: the very similar origin and insertion at the femur and calcis, giving a similar range of movement.

If now the lengths of the fibres of the plantaris and soleus are compared (fig. 2) the elements of the soleus are seen to be even shorter than those of the gastrocnemius and plantaris, measuring only 34 mm. in the specimen studied. This may be correlated with the origin of the soleus from the bones of the leg, so that it acts only on the ankle joint and not on the knee as well.

It is clear that the short length of the fibres is mechanically the most economical arrangement possible, for the range of movement between the origins and insertions of the muscles is limited by the nature of the bones and joints to which they are attached, and the fibre length is adequate to bring this range of movement about. Should the length of any fibre be doubled, then the extra half of the fibre would never be able to contract at all. In the

case of a muscle whose origin and insertion can move to and fro through a long distance, the fibres of that muscle are correspondingly long, as in the sartorius of Man.

This correlation of fibre length with mobility is confirmed by a study of a club foot (fig. 3) showing an abnormally limited movement of congenital origin. The lengths of the fibres are rather less than one-third those of the normal specimen (fig. 4), corresponding to the very small amount of movement allowed in such feet.

In strong contrast to the condition of the muscles in this congenital immobility stands the condition in an amputation stump resulting from an operation carried out in adult life (fig. 5). The tendo Achillis is firmly bound to the lower end of the tibia by new fibrous tissue, so that the gastrocnemius passing from femur to tibia could still act on the knee joint, but the soleus, now passing from one part of the tibia to another part of the same bone could have no action whatever. Yet in this condition of acquired immobility it is still fleshy and the fibres are of normal length. Thus it is clear that a process of growth is necessary for the fibres of a muscle to become suited in length to the work they perform. If the same amputation had been performed on a boy, the resultant adult specimen would show no muscular substance in the soleus.

The growth of muscle and tendon is an ill-understood subject. In the lower Vertebrates it is thought that the muscle is of myotomic origin, whereas the tendon is developed *in situ* from the mesenchyme of the developing limb bud. In the higher Vertebrates the myotomic origin of the limb muscles is less certain, and muscle and tendon are often held to be independent in development. It is noteworthy, however, that failure of junction of a muscle with its tendon is quite unknown, whereas it would be expected on the theory of independent development that such failure would occur as often as, say, failure of junction of the parts of the face. The absolute absence of any such anomaly suggests a closer relation between the developing muscle and tendon.

It may be noted that the combined length of the muscle and tendon is always such that at any age and in any individual the muscle is slung between its origin and insertion, so that it is neither taut nor loose. In the short muscular Bushman (fig. 6), for instance, the muscles are arranged just as efficiently as in the tall spare Bantu (fig. 1), so that they are neither torn when stretched nor slack when contracted, and this applies even to the anomalous muscles of the Bushman (fig. 6, *X* and *Y*).

Now this condition might be brought about by a system of genes in the individual cells of the bones and muscles, causing each part to be modelled in proportion to the others, or by a system of nervous impulses or hormones, keeping each part in touch with the parts around it. But either of these mechanisms would be much too complex to work in practice, particularly with the invariable perfection seen even in the case of anomalies (fig. 6) and congenital disturbances (fig. 3), so a more simple mechanism must be sought, one that will apply to all muscles and one that cannot go wrong.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1. Plantaris and gastrocnemius of Bantu.
Fig. 3. Anterior tibial muscles in a case of club foot.

Fig. 2. Plantaris and soleus of Bantu.
Fig. 4. Anterior tibial muscles of Bantu.

It is well known that the skin overlying a tumour becomes stretched, and this implies growth by traction, not only of the skin itself but also of the arteries and nerves supplying it. Growth of muscle is of precisely the same nature; that is to say, it grows in response to traction at its origin and insertion. This throws the control of the lengths of the muscles of the limbs on to the bones to which they are attached. If the bones grow, the muscles are stretched and grow to a length compatible with them.

This is not the only mechanism at work in muscle growth, for it does not explain the phenomena of muscle creep or muscle migration, but it is the most important mechanism, especially in the limbs, where migration is at a minimum.

The late foetal musculature is very similar to that of the adult, but there is relatively more muscle and less tendon. An extreme case of this is found in the serratus posterior superior of the eight months' foetus (fig. 7, *S.P.*) compared with that of the adult (fig. 8). Assuming that the adult in question passed at eight months through a stage comparable to that of the foetus shown, the amount of tendon is immensely increased. The part of the muscle inserted on the second rib has increased in fibre length from 20 to 30 mm., but the part inserted into the fourth rib has actually decreased from 20 to 17 mm. This diminution suggests that the tendon is lengthened at the expense of the muscular substance. Whether the tendon is formed from the muscle fibres themselves or from the connective tissue surrounding them cannot be decided by macroscopic examination, but in either case muscle is replaced by tendon.

Of the growth of tendon, once formed, little is known. There is some evidence that it can decrease in length, for no other explanation can be given of the condition of the soleus in the amputation stump (fig. 5) where the lower muscular fibres have been reversed in direction so that they now pass proximally from their origin to their insertion, apparently owing to contraction in the aponeurosis of insertion.

But of any interstitial increase in length, that is an increase otherwise than by accretion at either end from muscle or periosteum, there is no evidence. Though this point can only be settled by experimental work, it seems highly probable that tendons are to be classed with those organs, such as the bones, nails, yolk of eggs, the lens of the eye, teeth, etc., that cannot increase by interstitial growth. It increases by metamorphosis of muscle only.

The stimulus to tendon formation appears to be the limitation of the range of movement of the muscle by the conformation of the bones and joints. How the muscle fibre becomes sensible to its own inability to contract and perform work throughout its entire length is unknown, but Fenn has shown that certain processes only occur in the muscle if contraction actually occurs, and not if the muscle is not allowed to shorten (isometric contraction) (1). It may be that the absence of one of these changes gives the necessary stimulus.

Thus starting with a muscle tendon complex in a developing animal of any age after movement has once begun, the length of the complex keeps pace with the bones to which it is attached by growing in response to the traction

Fig. 1. Plantaris and gastrocnemius of Bantu.
Fig. 2. Plantaris and soleus of Bantu.
Fig. 3. Anterior tibial muscles in a case of club foot.
Fig. 4. Anterior tibial muscles of Bantu.

Fig. 5. Plantaris and soleus in an amputation stump.

Fig. 6. Calf muscles of Bushman.

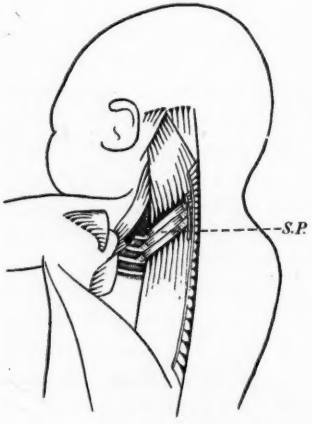

Fig. 7. Serratus posterior superior of eight months' foetus.

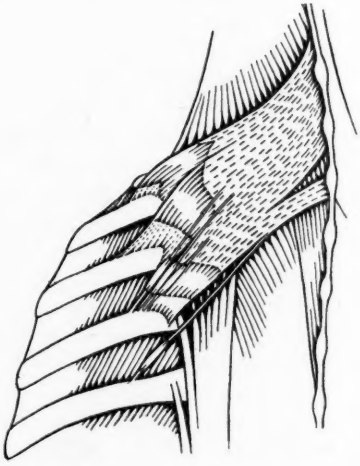

Fig. 8. Serratus posterior superior of adult.

set up within it, but growing only in its muscular part. This growth of muscle substance is counterbalanced by tendon formation in those parts which fail to contract owing to the limitation of possible movement.

There has been one previous important attempt to correlate development with function—that of Julius Wolff (3)—who claimed that the osteoblasts of a developing bone build according to the stresses and strains to which they are subjected. This noble theory was proved invalid by a study of the bones in foetal life, where the forces are different to those in the adult, but the bones show much the same structure, and by the fact that the theory implied interstitial growth in bone. The grounds for a mechanical explanation of the growth of muscle and tendon appear somewhat more secure, as the foetus is known to carry out muscular movements from an early age.

A neat example of the working of these theories is seen in the coccygeus muscle and the sacro-spinous ligament (fig. 9). It is generally acknowledged that the ligament is formed from part of the muscle, but no accurate description of the metamorphosis has been offered.

The ligament, however, is found to pass from the ischial spine to the immovable sacrum, while the muscle passes from the spine to the mobile coccyx. The response of the fibres to the limitation of movement between spine and sacrum has been complete tendinification.

In the lower animals, for instance in the baboon (fig. 10), the muscle has no insertion on the sacrum, which here consists of but three pieces. All the fibres pass to the basal vertebrae of the tail. The fibres are, as would be expected on the traction and limitation hypotheses suggested above, entirely muscular. Should any ape be found in which the coccygeus reaches the sacrum, that ape will be found to possess a sacro-spinous ligament.

The application of these theories to the field of Vertebrate evolution are immense. In the specialisation of limbs which has played a part in so many orders of Mammals, including that in which Man himself is to be found, it is no longer necessary to postulate complex co-ordinating mechanisms to govern the sizes of the muscles, nor a vast series of genes to suit the muscles to their work. Given in the developing organism a basic plan of musculature, the effects of traction and limitation will suit each mass to the cursorial limb of a horse, the specialised limb of a rabbit, or the familiar leg of Man (figs. 4, 9).

One point that has hitherto clouded an appreciation of the structure of muscles is the confusion between connective tissue and tendon; that is, between fascia and aponeurosis. If the representation of the anterior tibial muscles in fig. 4 is compared with that in any ordinary text-book of anatomy (2), it will be noted that the muscles are shown in my figure taking origin by a common superficial aponeurosis (fig. 4, *A*.) formed by their conjoined tendons of origin from the heads of the tibia and fibula. This structure, which may be named the aponeurosis cruris, is removed in ordinary dissection as "deep fascia" and is so represented in the text-books. But, being tendinous, it cannot be homologous to the deep fascia which covers the gastrocnemius or the

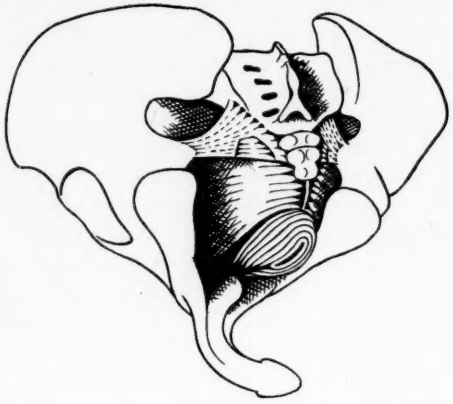

Fig. 9. Coccygeus muscle and sacrospinous ligament of Man.

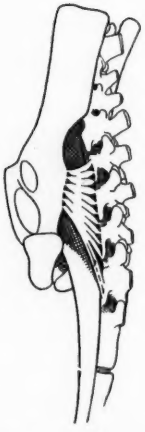

Fig. 10. Coccygeus muscle of baboon (*Papio*).

Fig. 11. Anterior tibial muscles of rabbit.

lower part of the leg. The true fascia lata is to be found as fibrous tissue covering the superficial surface of the aponeurosis in the same way as the areolar deep fascia of the thigh covers the subjacent muscles.

Similar structures are to be found wherever muscles are commonly said to arise from or to be inserted into fasciae or intermuscular septa, both of which are built up of connective tissue and are not constituted to take the strains of muscle pull. Every muscle fibre, apart from those belonging to the skin musculature, such as those of the face, perinaeum, etc., either directly or through tendon, takes origin from bone and is inserted into bone.

SUMMARY

It is attempted along morphological lines to substantiate the following laws of muscle and tendon growth:

(1) That the length of the fibres of any muscle bears a constant proportion to the distance through which its origin and insertion can be approximated.

As corollaries to this:

That muscles contracting through the same distance have fibres of equal length, and

That all the fibres of a single narrow muscle are of the same length.

Bringing about this correlation:

(2) That muscle fibres grow along their whole length in response to *traction* set up by the growth of the bones to which the muscle is attached.

(3) That tendon is lengthened by metamorphosis of muscle tissue in response to a *limitation* of the range of possible contraction determined by the nature of the attachments of the muscle.

As a result of these mechanisms:

(4) That muscle can adapt itself to the changing shapes of limbs in an evolving phylum without any necessary alteration in the genetic make-up of the constituent species.

I must thank the anatomists of University College, in particular Profs. Elliot Smith and Harris, for the groundwork; Profs. Woollard and Watson for suggestions; Prof. M. R. Drennan for his encouragement and active interest in my work, and for much of the material, and finally the Research Grant Board of the University of Cape Town for defraying all the expenses.

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THE EARLY DEVELOPMENT OF THE FERRET: THE ZONA GRANULOSA, ZONA PELLUCIDA AND ASSOCIATED STRUCTURES[1]

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INTRODUCTION

During the last few years interest in the zona granulosa and zona pellucida of mammalian ova has waned. This is not unnatural, for it appears as if nothing very new could be learned, at least from paraffin sections, regarding the structure and functions of these membranes. The zona granulosa is generally regarded as a means whereby the ovum, at least in the ovary, is nourished (Marshall, 1922). The zona pellucida is a protective coat, and in particular its late disappearance seems (Robinson, 1904) to be associated with the late implantation of the ovum.

To show that there is still something to be gained from the study of ordinary sections is partly the object of the present record. Moreover, most of the detail regarding these membranes has been obtained from a study of non-Carnivores. The present study is of a Carnivore—the ferret.

MATERIALS AND METHODS

The material used in these investigations was the same as that employed in the observations on the ferret pronuclei (Mainland, 1930) and on the cytoplasm of ferret ova (Mainland, 1931), viz., 101 ferret ova at stages from the second polar spindle to the first segmentation spindle inclusive. The ova were in the form of mounted paraffin sections cut at 10 microns. The fixatives that had been used were Flemming, Mann with and without formol, Perenyi, Zenker, and the staining methods for different ova were Heidenhain's iron-haematoxylin and Mayer's haemalum, with either eosin or orange G as counterstain.

The investigations have involved the use of a method that the author feels more and more to be essential wherever there are variations in phenomena, and, perhaps more than usual, where the number of specimens available is limited. This method is the application of biometrical tests, particularly the χ^2 test. In a study of the pronuclei (Mainland, 1930) this method was discussed. Fisher (1930) gives full details, with tables showing the significance of different values of χ^2. It should be pointed out here that an association between phenomena is said to be "significant" when the odds are 19 or more to 1 against the association being due to chance.

[1] Part of a thesis recently accepted by Edinburgh University for the degree of Doctor of Science.

ZONA GRANULOSA

The records of Robinson (1918) have shown the resemblance between the zona granulosa of the ferret and that of other Mammals. It may now be subjected to a more minute examination. Out of a total of 95 ova at the early tubal stage (i.e. at the stages of pronuclei, second polar spindle or first segmentation spindle), 86 showed more or less of the zona granulosa of the follicle from which they had come. The remaining 9 showed no cells of this kind near them.

Amount of zona granulosa

As is well known, the cells of the zona granulosa disappear as the ovum travels down the uterine tube. It appears desirable to demonstrate the rapidity with which this disappearance occurs, and this is of special importance if, as is strongly indicated (see below), the zona granulosa is associated with the nutrition of the tubal ovum. Table I shows the relative amounts of zona granulosa found near the different ova, arranged according to the stage of development of the ovum. These data were grouped to form a fourfold table, and

Table I. *Association between amount of zona granulosa and condition of nuclear material in ovum.* (*The numbers of ova are recorded.*)

	Amount of zona granulosa					
Nuclear material	Nil	Very small	Small	Medium	Abundant	Total
Second polar spindle	—	—	—	—	15	15
Pronuclei peripheral or subcentral	4	—	3	7	1	15
Pronuclei central	5	5	13	11	5	39
First segmentation spindle	—	—	1	—	—	1
Total	9	5	17	18	21	70

the results of the application of the χ^2 test were: $\chi^2 = 13{\cdot}6$, $n = 1$. The results were therefore very highly significant, showing that the disappearance of the zona granulosa can be definitely detected as the ovum advances from the stage of second polar spindle to the stage of first segmentation spindle. Thus there is given a criterion of development apart from the condition of the ovular nuclear material.

A further test was applied to show the relation between the number of hours after insemination of the animal and the amount of zona granulosa remaining near the ovum. It has been shown (Mainland, 1930) that the post-inseminal period is to some extent a measure of the ovular development, for the more developed ova were in the main found in those animals that had lived longest after insemination. There was, however, no significant association between the length of the post-inseminal period and the amount of zona granulosa present. The post-inseminal period, as is well realised, is in the ferret not a very satisfactory measure of development, owing probably to the

great variation in the lapse of time between insemination and ovulation. In this series, therefore, there could not be demonstrated any change in the amount of zona granulosa in the first 100 hours after insemination.

Staining and shape of cells

Little difference was noted in the eosinophilic staining of the cells as development proceeded. More instructive results were obtained from examination of the shape.

When the ovum is liberated it has around it the typical corona radiata, with an inner layer of pyramidal cells and two or three outer layers of polyhedral cells. Illustrations are to be found in Robinson's monograph (1918) and also in the accompanying figure (Plate I, fig. 7). As the ovum proceeds down the tube the zona granulosa cells degenerate. This degeneration, like the degeneration of many of the cells of the ovary, deserves attention, for it takes place in a healthy environment. It occurs in a medium composed partly of follicular fluid, partly of fluid from the tube wall and partly, no doubt, of

Table II. *Association between shape of zona granulosa cells and condition of nuclear material in ovum.* (*The numbers of ova are given. The table is condensed.*)

	Dominant shape of zona granulosa cells			
Nuclear material	Pyramidal	Polyhedral and irregular	Spherical and ellipsoidal	Total
Second polar spindle	9	—	—	9
Pronuclei peripheral or sub-central	2	1	8	11
Pronuclei central or first segmentation spindle	3	12	16	31
Total	14	13	24	51

fluid from the developing ovum itself. The degenerating cells are of various shapes (Plate I, fig. 2). Some are polyhedral, others more irregular, others spherical and still others ellipsoidal. Table II shows the relationship between the stage of ovular development and the shape of the zona granulosa cells. The χ^2 test was applied to the data grouped in a 3×2 table, and proved that there was a significant relationship between the shape of cell and the ovular development. Inspection of Table II reveals that the significance of the association is largely if not wholly due to the greater frequency of the pyramidal cells at the polar spindle stage. It was interesting to note that when the post-inseminal period was used as a measure of development, there was a highly significant association between the shape of the zona granulosa cells and the development as thus indicated. This was owing to the greater abundance of pyramidal granulosa cells around the ova at the second polar spindle stage, these ova being found in the animals with the shortest post-inseminal period. Thus the post-inseminal period appears on some occasions a satisfactory indication of development.

The next problem is the elucidation of the order of shape changes. There is no indication from the figures that spherical cells occur more abundantly at the later than at the middle stages of development, and therefore the stages might be: pyramidal, polyhedral, spherical; or: pyramidal, spherical, polyhedral. Examinations of the specimens suggested that the former series was correct. A definite answer to the question was obtained by studying the relationship between the amount of granulosa and cell-shape. It was obvious that, as would be expected, when the shape of cell was pyramidal, the amount was greater than when the cells were of other shapes; but even when all the specimens with pyramidal cells were excluded it could be shown by the χ^2 test that the less abundant the granulosa the more often were its cells spherical or ellipsoidal. It appeared as if more and more of the zona granulosa cytoplasm disappeared and the remainder became a spherical shell around the nucleus.

There was no association demonstrable between differences of fixative and shape of cells.

Nuclear structure

The zona granulosa of each ovum was classified according to whether its nuclei showed only globules (or granules) of chromatin or whether some or all of its nuclei were homogeneously basiphilic (Plate I, figs. 2–4). It seemed as if the basiphilic nuclei were a sign of degeneration, but to test this supposition use was made of the fact just recorded that the shape of the granulosa cells changed from pyramidal to polyhedral and then to spherical. It was then shown that there was almost, but not quite, a significant association between nuclear structure and cell-shape. The nuclei, therefore, are of little value as an index of degeneration. There was no significant association between the nuclear structure and the stage of development of the ovum as indicated by its polar spindle or pronuclei.

The caution which is necessary in applying a χ^2 test is indicated by the results obtained when the association between the post-inseminal period and the nuclear condition of the granulosa was investigated. This test showed a highly significant association between the two, that is, more granular nuclei were found associated with the ova early after insemination than later on. This association appeared difficult to explain, since, as has just been stated, there was no apparent association between ovular development and the condition of the granulosa nuclei. The explanation was revealed, however, when the effect of staining was observed. For each section a record was made of the depth of the basic staining of the ovum and the surrounding tissues, e.g. tube and ovary, the classification being "dark," "medium," "pale." It was then shown that there was a highly significant association between the condition of the granulosa nuclei and the depth of staining. The homogeneous nuclear appearance was much more common among deeply stained sections than among the less deeply stained. Moreover, it was found that, for some reason or another, deeply stained sections were much commoner among the ova taken at the late

post-inseminal periods. This would account for the result mentioned above. The more deeply stained sections had more homogeneous nuclei. These sections were more abundant at the later post-inseminal periods, and therefore a spurious association was produced between granulosa nuclear structure and the post-inseminal period.

Since the relation between depth of staining and homogeneity of the nuclei is so strong, one cannot help feeling that this may mask a relationship between the nuclear structure and the degeneration of the cells. This suggestion was, however, not confirmed by any tests. A test, for example, was made by using the amount of granulosa present as an index of its progress towards degeneration. There was no significant association between this amount and the nuclear structure. The conclusion is that, so far as can be shown, the nuclear structure is not an index of the degeneration.

The χ^2 test showed no significant association between the fixative (Mann, Zenker or Perenyi) and the nuclear structure of the granulosa cells.

Relationship of zona granulosa and ovum

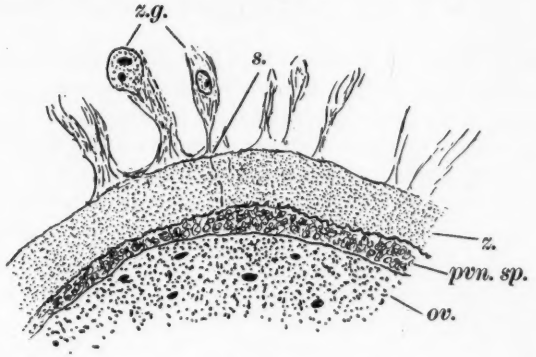
In 10 ova of the collection some of the cells of the granulosa demonstrably penetrated the zona pellucida (text-fig. 1). In these specimens the granulosa was in the form of a corona radiata. In 9 other ova, in which the zona granulosa also formed a corona, no penetration of the zona pellucida was detected. Owing to the smallness of the numbers no conclusion could be reached regarding the influence of fixative and staining on the demonstration of this penetration. In some specimens the penetration when visible was not through the whole thickness of the zona pellucida.

The interest of the proximity of the zona granulosa to the ovum lies partly in a consideration of the factors that influence the disappearance of the granulosa—dissolution by degeneration, removal by the cilia of the tubal wall and removal by the muscular contractions of the tubal wall, if, as Sobotta (1922) considers to be the case, the muscular activity of the tube is the cause of the passage of the ovum. There was a definite association between the amount of granulosa near the ovum and its proximity to the ovum, i.e. whether it was in close contact, at a little distance or at a greater distance. This was to be expected if the removal of the granulosa is largely mechanical. If degeneration alone were responsible for its disappearance, it would be expected that the cells would be near to the ovum throughout the whole course of the degeneration. They do not degenerate to the stage of forming an amorphous mass before being removed.

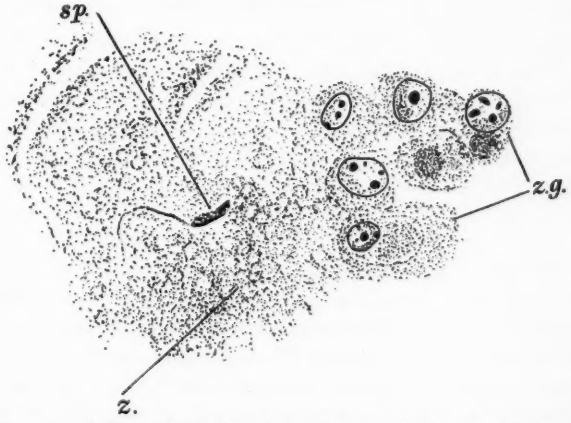
STRUCTURE OF ZONA PELLUCIDA

Since these investigations on the ferret were confined to ova after ovulation, no attempt is made to demonstrate the origin of the zona pellucida. Its structure and staining reactions, however, deserve attention, because of the different theories of its formation and also because of the "zona pellucida bodies"

discussed below. In the attempt to elucidate this question it was necessary to consult the various records of zona pellucida development. Among other records are those of Cattaneo (1922), Dubreuil and Regaud (1908), Fischer (1905), O. van der Stricht (1923) and Mjassojedoff (1923).



Text-fig. 1. Ovum no. 24. At point *s.* a strand of a zona granulosa cell penetrates the entire thickness of the zona pellucida, *z.*; *z.g.*, zona granulosa; *pvn. sp.*, perivitelline space containing granular material; *ov.* ovum. (Mann with formol; Heidenhain's iron-haematoxylin and orange G; camera lucida drawing; magnification approx. 1000.)



Text-fig. 2. Ovum no. 2. Tangential section through zona pellucida, *z.*, showing the pitted or "cellular" appearance of its outer part; *z.g.*, zona granulosa; *sp.*, spermatozoon in zona pellucida. (Zenker; Heidenhain's iron-haematoxylin and eosin; camera lucida drawing; magnification approx. 1000.)

Texture of zona pellucida

Tangential and oblique sections through the zona pellucida demonstrated the pitted or "cellular" structure of its outer part (text-fig. 2). This was demonstrated by haematoxylin, but was observed also in specimens merely

tinted with eosin. It was independent of the difference in fixatives. On transverse section the zona pellucida was smooth at its inner surface, rather more rough at its outer surface, in agreement with the cellularity observed on tangential section.

Staining

Heidenhain's iron-haematoxylin with a light eosin counterstain gave a grey, bluish grey, blue-black or deep purplish colour to the zona pellucida. If the basic stain was less deep as in the ova stained by Mayer's haemalum, the eosin gave the membrane a dull pink stain or an orange colour. When the specimens (fixed in Perenyi's fluid) were stained with Heidenhain's iron-haematoxylin and counterstained with orange G, the outermost part (say 1/5 or 1/6) of the zona pellucida was pale yellow, the intermediate part (the largest portion) was of a darker yellow colour, while the innermost part was a dark grey line. This suggests a difference in texture of the different parts, especially a lack of density in the parts adjacent to the zona granulosa.

Specimens fixed in Mann's or Zenker's fluid and stained with Mayer's haemalum and eosin showed in some cases a purple outermost zone while the rest of the zona pellucida, larger in extent, was orange. Similarly, among 4 Flemming-fixed specimens, 2, stained moderately deeply with Heidenhain, showed the outer part of the zona pellucida blue-black, the inner part yellowish grey.

There was no difference in staining between the Perenyi and Zenker specimens when stained with Heidenhain's iron-haematoxylin, nor between Zenker and Mann specimens stained with Mayer's haemalum and eosin, nor between Perenyi specimens stained with Heidenhain and orange G and those fixed in Mann's fluid and formol and stained in the same way.

Striae

Under the subject of zona granulosa reference is made to the presence of strands of the corona radiata penetrating the zona pellucida. Except for these, no striae were seen passing through the entire thickness of the zona pellucida. On the other hand the majority of the specimens, whatever the fixative or stain, showed numerous fine radial striae in the peripheral part of the zona pellucida (Plate I, fig. 5). Even Flemming specimens tinted with eosin showed these. They were present irrespective of the presence or absence of corona radiata. They presumably corresponded to the attachments of the pointed ends of the corona radiata cells.

Size

Exact determination of the thickness of the zona pellucida was not made. It is indicated in the figures of ova accompanying the text, and may be stated as roughly 4–6 μ.

ZONA PELLUCIDA BODIES

Occurrence and structure

In the zona pellucida of 16 out of 99 tubal ova there were discovered spheroidal or ellipsoidal bodies (Plate I, figs. 1 and 2). These bodies stained usually more darkly than the zona pellucida, but rather similarly to this structure, i.e. grey or bluish grey or dark blue in the specimens stained by Heidenhain's iron-haematoxylin and a little eosin, and a dull red in the ova stained by Mayer's haemalum and strongly counterstained with eosin. Their structure was homogeneous except that a few had each a central homogeneous basiphil globule. It appeared unnecessary to determine very exactly the size of these bodies. Their diameters were frequently of about the thickness of the zona pellucida. In some specimens they were less than even the nuclei of the neighbouring granulosa cells.

The 16 ova that showed these bodies were from 4 different animals (1 tube from each animal). Other ova from the same animal or tube did not necessarily show such bodies.

Association with fixative and stain

These bodies were found only in specimens fixed in Zenker's fluid. Thus out of 26 specimens fixed in Zenker and stained by Heidenhain's iron-haematoxylin and eosin, as many as 12 definitely showed such bodies and 2 showed them doubtfully. Out of 15 specimens fixed in Zenker and stained by Mayer's haemalum and eosin, 4 showed such bodies. Similar bodies were not found in the 23 specimens fixed with Perenyi's fluid and stained with Heidenhain, nor in the 19 Mann-fixed specimens, nor in the 15 Flemming-fixed specimens. The association with Zenker's fixative is therefore of extremely high significance.

With regard to staining it may be pointed out that the central basiphil globule was present only in the zona pellucida bodies in specimens stained with Heidenhain's iron-haematoxylin, and not in all of these. In this connection it should be recalled that the zona pellucida itself can, as mentioned above, take on a deep basic stain, and there is no need to suggest that the basiphil centre of the zona pellucida bodies is of the nature of a nucleus.

There was no significant difference between the power of Heidenhain's iron-haematoxylin and Mayer's haemalum to demonstrate the zona pellucida bodies in Zenker-fixed ova.

The demonstration of these bodies by the use of Zenker's fluid and the paraffin section technique shows that they do not consist wholly of lipoid material. The failure of Flemming's fluid to demonstrate them shows that if any lipoid is present in them, it must have less than 50 per cent. non-saturated fatty acid (Parat, 1927). They are presumably of protein origin. The difference between the effects of Zenker's fluid, which demonstrates them, and Perenyi's fluid, which does not, is more difficult to explain. Zenker's fluid resembles Perenyi's in possessing a chromic acid compound, but Zenker contains also

mercuric chloride, which may be the cause of the demonstration of the bodies. The fact that one protoplasmic fixative and not others demonstrates them suggests that they are products of the cytoplasm rather than cytoplasm themselves. They may be either degeneration products or secretory products.

Association with bodies in zona pellucida of ovarian ova

In the preparation of most of the specimens the tube and ovary had been cut and mounted together and consequently the slides containing the tubal ova contained in many cases also sections of medium-sized or even large ovarian ova. Out of the 16 instances in which there were bodies in the zona pellucida of tubal ova, there were 11 in which medium or large ovarian ova showed similar bodies. There was shown to be a very definite association between the two occurrences.

Association with bodies in cytoplasm

As mentioned above, 15 ova had been fixed in Zenker's fluid, stained by Mayer's haemalum and strongly counterstained with eosin. Out of these, 13 showed bodies in the cytoplasm resembling in size and staining qualities the zona pellucida bodies. The relationship between the occurrence of these cytoplasmic bodies and the zona pellucida bodies is shown as follows (the numbers representing ova):

	Bodies in zona pellucida		
	Present	Absent	Total
Bodies present in cytoplasm	3	10	13
Bodies absent from cytoplasm	1	1	2
Total	4	11	15

None of the other ova, fixed either in Zenker's or other fluid, showed bodies that were at all comparable to these cytoplasmic bodies. There was therefore a significant association between the occurrence of the zona pellucida bodies and that of the cytoplasmic bodies, inasmuch as they only occurred after Zenker fixation. There was however a definite difference between them in relation to staining. Mayer's haemalum and deep eosin staining demonstrated the cytoplasmic as well as the zona pellucida bodies, whereas Heidenhain's iron-haematoxylin demonstrated only the zona pellucida bodies. Among the Zenker-fixed ova stained by Mayer and eosin there was a significantly greater number of ova with cytoplasmic bodies than with zona pellucida bodies.

The relationship of the cytoplasmic bodies to the zona pellucida bodies will be discussed later. At present it is desirable to give more detail regarding the structure of the cytoplasmic bodies (Plate I, fig. 6). Thus, in ovum No. 55 there lay in the cytoplasm near its edge an irregular ellipsoidal body stained orange with eosin. The surface facing the zona pellucida was more irregular in contour than the surface facing the centre of the ovum. In another part of the same ovum were three similar bodies. One of these, nearer the zona pellucida, had a

smooth surface facing the zona pellucida; the two deeper in the ovum were more irregular all round and less homogeneous in texture. The irregularities of these bodies corresponded fairly closely to the alveolar spaces of the cytoplasm itself, spaces which in the living ovum were filled with lipoid material. If the bodies can so accommodate themselves to the masses of deutoplasm, it appears that the cytoplasmic bodies must be fairly fluid before fixation.

Differences in size between the bodies in the peripheral part of an ovum and those in the central part were not marked, and there was no evidence of absorption in the sense of a diminution in the size of the bodies as a result of a dissolution progressing from the edges towards the centre.

Table III. *Association between amount of granulosa and presence of zona pellucida bodies.* (*Zenker specimens only. Numbers of ova.*)

	Zona pellucida bodies				
Amount of granulosa	None	Doubtful	Present	Many	Total
Nil	3	—	—	—	3
Very small	1	—	3	—	4
Small	8	—	—	—	8
Medium	6	—	6	3	15
Large	3	1	4	—	8
Total	21	1	13	3	38

Table IV. *Association between amount of granulosa and presence of bodies in cytoplasm.* (*Numbers of ova.*)

	Cytoplasmic bodies		
Amount of granulosa	None or doubtful	Present or numerous	Total
Small	3	7	10
Medium or abundant	9	6	15
Total	12	13	25

Association with zona granulosa

The Zenker-fixed ova were arranged according to the presence or absence of the zona pellucida bodies and according to the amount of zona granulosa present (Table III). There was shown by the χ^2 test to be a significant association between the two classifications. Ova with a medium or large amount of granulosa near them more often presented zona pellucida bodies than did ova where the zona granulosa was absent or small in amount. Two possible interpretations might be placed upon this. It might be claimed as a proof that the zona pellucida bodies were products of the zona granulosa; or the amount of granulosa might be taken simply as an indication of the development of the ovum (see above), and then the relationship of granulosa and zona pellucida bodies would indicate that these bodies disappeared as the ovular development progressed.

It should be noted at this point that there was no significant association between the occurrence of the bodies in the cytoplasm and the amount of zona granulosa near the ova. If anything there was a tendency for these bodies to be more often found where the zona granulosa was small (Table IV). There was therefore a distinct difference between the zona pellucida bodies and the cytoplasmic bodies in this respect. It was thought possible that this was a spurious difference due to the difference between Mayer's staining and Heidenhain's in demonstrating the bodies in the cytoplasm, but χ^2 tests showed no indication that this was so. The conclusion is therefore that, while the zona pellucida bodies get less frequent as the ovum progresses and the zona granulosa disappears, the bodies in the cytoplasm remain, or perhaps even increase in frequency.

Association with bodies outside the ovum

In 15 specimens spheroidal bodies resembling those of the zona pellucida in shape, size and staining reactions were discovered outside the ovum, some in contact with the zona granulosa near the tubal ovum, some in the tube at a distance from the ovum. These bodies appeared fairly homogeneous in structure. Similar bodies were found in the liquor folliculi of some moderate sized follicles in the ovary corresponding to ovum No. 65 which itself showed zona pellucida bodies. In one specimen corresponding to ovum No. 67 somewhat similar bodies were found among the granulosa cells of a recently ruptured follicle, i.e. one about to become a corpus luteum.

Relationship of zona pellucida bodies to perivitelline space

In one ovum a zona pellucida body projected into the perivitelline space causing a depression of the adjacent surface of the ovum. In ovum No. 11 (Plate I, fig. 8) several bodies were seen partly inside the zona pellucida, partly outside, while some lay in the perivitelline space. The part against the ovum did not depress the latter, but was spread out on its surface. The staining of the bodies in the space was sometimes a little lighter, sometimes a little darker than that of the zona pellucida itself. There was no evidence that the zona pellucida was deficient near the bodies that lay in the space. The zona pellucida did not seem to have lost any of its substance although the bodies so closely resembled it in staining reactions.

The above data have been recorded without the introduction of any suggested explanation. The question of origin and destiny may now be discussed.

Origin and destiny of zona pellucida bodies

Before proceeding with an attempt to elucidate the genesis and fate of these bodies, it is desirable to note the references to closely similar structures in other animals. The author is acquainted with only two such descriptions. Sobotta (1908) recorded the occasional appearance of small nucleated cells,

not polar bodies, in the perivitelline space of mouse ova at the early tubal stages of development, but these bodies could not be classified definitely as follicular epithelial cells or as abnormalities in the structure of the zona pellucida. A few years later Sobotta and Burckhard (1911) described in regard to the tubal ova of the rat some roundish bodies readily stained with acid aniline dyes. These bodies were sometimes in the zona pellucida, sometimes between the zona granulosa cells, and commonly appeared first in the later stages of fertilisation. The authors could not decide whether the bodies were products of tubal secretions or of hyaline degeneration of the zona granulosa cells. At first sight it appears rather remarkable that a structure that is so common in the tubal ova of the ferret should be so seldom mentioned in connection with the ova of other animals, but technique may well be the cause of this. O. van der Stricht and his collaborators, for example, were so much concerned with cytoplasmic structure that Zenker's fluid was seldom used in their preparations, and it has been shown above that this fixative is the only one that demonstrated the bodies in the present investigations.

A brief recapitulation of the facts so far demonstrated in the ferret may now be made.

The results of fixation strongly suggested that the zona pellucida bodies were not cytoplasmic in structure, but were rather the products of degenerated or secretion products. Similar bodies were found in relation to ovarian ova, and were demonstrated by Mayer's haemalum and eosin in the cytoplasm of tubal ova. The zona pellucida bodies were less frequently met where the amount of zona granulosa near the ovum was small. There was if anything a tendency in the opposite direction with regard to the cytoplasmic bodies. In a number of instances structures somewhat similar to the zona pellucida bodies were found outside the tubal ova among the granulosa or elsewhere in the tube.

The bodies in the perivitelline space suggest stages of a progression into or out of the zona pellucida and out of or into the ovum. No adequate transition stages were observed between the cytoplasmic bodies and the zona pellucida bodies, but there appeared no reasonable ground for doubt of the identity of these two. It is, of course, obvious that, since they were found near the ovarian ova they could not be products of the uterine tube. The question next arises whether the bodies are products of the ovum or of the zona granulosa, i.e. the question of direction in which the bodies are passing. This is partly answered by the fact that as the zona granulosa cells get smaller in number, i.e. as the ovum advances in development, the zona pellucida bodies are less frequently met, while the cytoplasmic bodies are as abundant, or perhaps even increase in number.

One of the ovarian ova corresponding to the tubal ovum No. 17 was very instructive in regard to the origin of the zona pellucida bodies. The specimen was a medium-sized healthy ovarian ovum, fixed in Zenker and stained with Heidenhain. In its zona pellucida small basiphil granules were detected, and by careful focussing it appeared possible to detect a connection between these

and the strands of the zona granulosa around the ovum. No other example of this was observed, but the numbers of available ovarian ova of fair size were small.

When the zona granulosa is in the form of degenerating rounded cells it is sometimes very close to the zona pellucida, and may be somewhat imbedded in the outer surface of the latter. It may therefore be asked whether the zona pellucida bodies and those found in the ovular cytoplasm are not degenerate granulosa cells. This suggestion is further supported by the presence in some of the zona pellucida bodies of a central area stained blue-black like a nucleus by Heidenhain's stain. On the other hand, this area was absent from most of the bodies, even when stained by this method. Moreover, while some of the bodies were large enough to be granulosa cells, others could at most be fragments of granulosa cells. The presence of bodies in relation to ovarian ova is also strongly against this possibility, and it should be recalled that the fixation phenomena indicated that they were not actually cytoplasm.

The strong resemblance between the bodies and the zona pellucida is striking and important, and the conclusion of these investigations is that the zona pellucida bodies are products of the zona granulosa, in a very similar fashion to part, at least, of the zona pellucida. The bodies are absorbed by the ovum.

There is thus indicated a function for the zona pellucida beyond that of protection. During the process of development of the ovum in the uterine tube the zona pellucida contains material that is probably utilised by the ovum and passes this material on to the ovum.

PERIVITELLINE SPACE

The space between the zona pellucida and the ovum proper varied in extent from ovum to ovum. It was impossible to say whether this variation was due entirely to differences in the amount of shrinkage during preparation of the ova. This space always contained at least a small quantity of a finely granular material similar to the neighbouring cytoplasm (Plate I, fig. 4). In some specimens, as in Plate I, fig. 5, the space was large and the granular material very definite. There seemed to be no reason to regard this granular material as a product of deutoplasmolysis. It could arise equally well from the precipitation of some albuminous periovular fluid.

PERIVITELLINE MEMBRANE

In the various photographs shown in Plate I the edge of the ovum proper will be noticed as marked by a line that is denser than the rest. No further evidence of the nature of this "perivitelline membrane" was obtained from a study of the sections. The most profitable way of studying this structure, like the structure bearing the same name in Invertebrate ova, is to investigate the fresh unfixed ovum in which, 50 years ago, van Beneden (1880) working on the rabbit demonstrated that the membrane could be isolated.

SUMMARY

Investigations have been made of the zona granulosa, zona pellucida and associated structures of 101 ferret ova in stained paraffin sections, biometrical tests being applied to show the significance of the results. The stages examined were from the second polar spindle stage to that of the first segmentation spindle.

In spite of the great variation between different ova, the disappearance of zona granulosa could be detected as the ova advanced from the first to the last of these stages. Probably owing to the great variation in the lapse of time between insemination and ovulation it was not possible in this series to demonstrate any change in the amount of zona granulosa near an ovum in the first hundred hours after insemination.

The eosinophilic staining of the cells did not change detectably as the ova advanced in development.

There was a significantly greater number of ova with pyramidal granulosa cells at the second polar spindle stage than later in development. The order of shape changes is indicated as: pyramidal, polyhedral, spherical.

There was no indication that the nuclear structure of the granulosa cells altered as the ova developed, or that the differences in nuclear structure indicated degeneration. The more intense the basiphilic staining of the sections, the more abundant were homogeneously stained granulosa nuclei.

The greater the amount of zona granulosa near an ovum, the nearer to the ovum did the cells tend to lie, as would be expected if the cause of their disappearance were mechanical removal rather than mere degeneration and dissolution.

The structure of the zona pellucida was studied (p. 590). The majority of the ova showed radial striae in the zona pellucida, but these were only in its outermost part except in those ova where cells of the corona radiata demonstrably penetrated far into or through the zona pellucida.

The thickness of the zona pellucida was roughly 4–6 μ.

Sixteen of the ova showed spherical or ellipsoidal bodies in the zona pellucida. These were demonstrable only in Zenker-fixed ova, not at all in Perenyi-, Flemming- or Mann-fixed ova. They were shown either by staining with Heidenhain's iron-haematoxylin or by staining with Mayer's haemalum. There was a significant association between the occurrence of these bodies and the occurrence of similar bodies in medium-sized or large ovarian ova. Similar bodies were found in the cytoplasm of 13 out of 15 Zenker-fixed tubal ova stained with Mayer's haemalum and eosin, but these cytoplasmic bodies were not demonstrated by Heidenhain's iron-haematoxylin.

The zona pellucida bodies got less frequent as the ova progressed in development and the zona granulosa disappeared, whereas the similar bodies in the cytoplasm remained or perhaps even increased in frequency.

In a number of instances structures somewhat similar were found outside the tubal ova in the tube and in the liquor folliculi of the ovary.

It was concluded that the zona pellucida bodies were products of the zona granulosa arising in a very similar fashion to part, at least, of the zona pellucida, and that the bodies were absorbed by the ovum. The zona pellucida is therefore more than a mere protection to the ovum.

The perivitelline space varied in extent in different ova. It always contained some finely granular material, not necessarily a product of "deutoplasmolysis."

The study of the sections did not give evidence of the nature of the perivitelline membrane.

ACKNOWLEDGMENTS

To Prof. Arthur Robinson of Edinburgh University I am very much indebted for the loan of the specimens on which these investigations were carried out. To the trustees of the Moray Fund I wish to express my thanks for financial assistance in the preparation of some of the specimens. The observations were carried out in the Anatomy Department of Manitoba University and the work was completed in Dalhousie University. To Miss Nason of the Pathology Department of Manitoba University I am much obliged for the preparation of the photographs.

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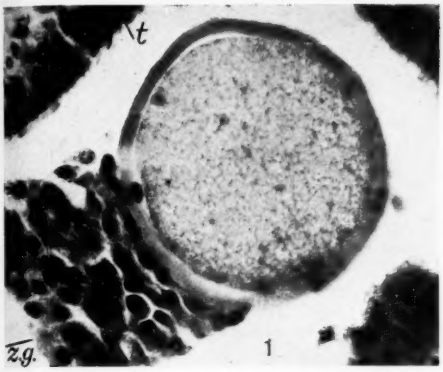

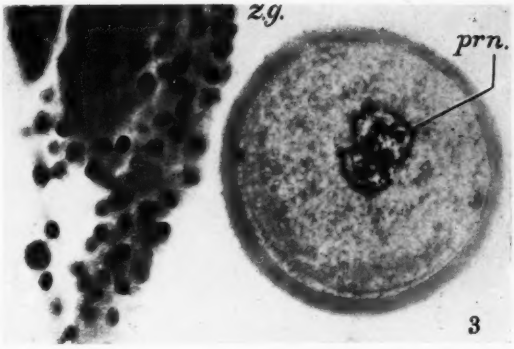

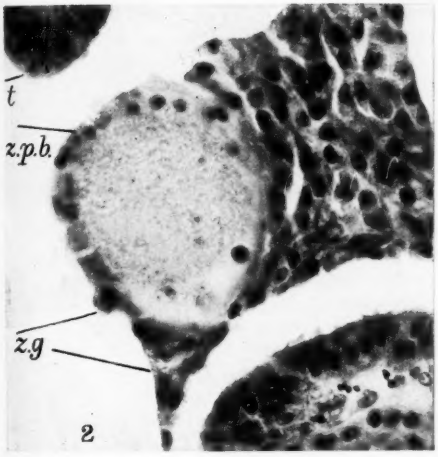

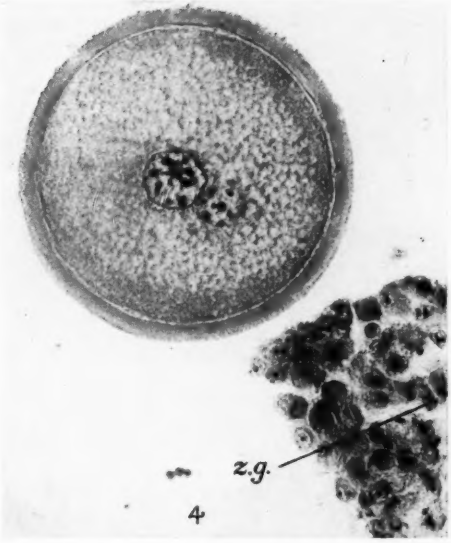

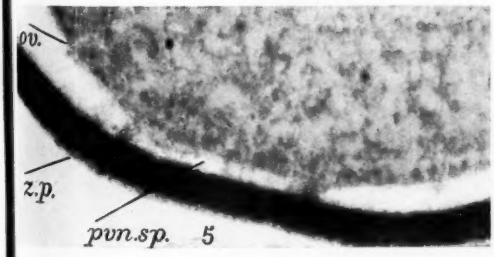

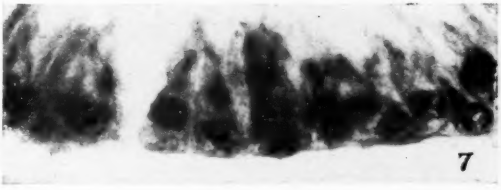

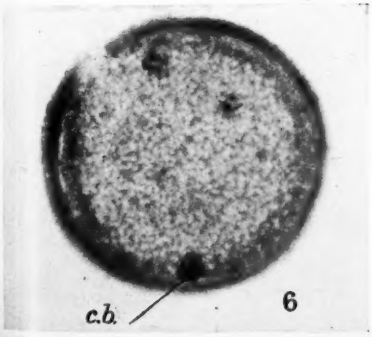

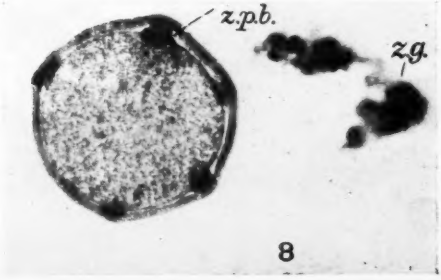

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EXPLANATION OF PLATE I

ABBREVIATIONS

c.b.	Cytoplasmic body.	*t.*	Tube (uterine).
ov.	Ovum.	*z.g.*	Zona granulosa.
prn.	Pronucleus.	*z.p.*	Zona pellucida.
pvn.sp.	Perivitelline space.	*z.p.b.*	Zona pellucida body.

Fig. 1. Ovum no. 17, showing zona pellucida bodies and zona granulosa. Zona granulosa nuclei are mostly homogeneously basiphil. (Zenker; Heidenhain's iron-haematoxylin and eosin; magnified by 375.)

Fig. 2. Ovum no. 17, a section nearer the end of the ovum than that of Fig. 1. Numerous zona pellucida bodies of various sizes; zona granulosa with polyhedral cells and many homogeneous nuclei. (Zenker; Heidenhain's iron-haematoxylin and eosin; magnified by 375.)

Fig. 3. Ovum no. 3. Granular matter in perivitelline space; zona granulosa cells massed together; many homogeneous nuclei. (Zenker; Heidenhain's iron-haematoxylin and eosin; magnified by 450.)

Fig. 4. Ovum no. 6. Zona granulosa with polyhedral cells; nuclei granular. (Zenker; Heidenhain's iron-haematoxylin and eosin; magnified by 425.)

Fig. 5. Ovum no. 93. Irregular areas in outer part of zona pellucida, less dense than the rest of it, suggesting radial striae; granular matter in perivitelline space. (Perenyi; Heidenhain's iron-haematoxylin and eosin; magnified by 1200.)

Fig. 6. Ovum no. 55. Bodies in cytoplasm, one appearing dense in the part facing zona pellucida, more alveolar in the part of it facing centre of ovum. (Zenker; Mayer's haemalum and eosin; magnified by 650.)

Fig. 7. Ovum no. 10. Zona granulosa of pyramidal cells, apices pointing towards zona pellucida. (Perenyi; Heidenhain's iron-haematoxylin and eosin; magnified by 750.)

Fig. 8. Ovum no 11. Zona pellucida bodies, two in zona pellucida, two in perivitelline space. The paler granular matter in perivitelline space is probably a polar body. Remnants of zona granulosa visible. (Zenker; Heidenhain's iron-haematoxylin and eosin; magnified by 275.)

FURTHER NOTES ON THE ARRANGEMENT OF THE COLLAGEN FIBRES OF VEINS, TOGETHER WITH CERTAIN OTHER OBSERVATIONS ON VEINS, AND ON THE VENOUS RETURN

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Some work which I began in 1930 on the venous return led to an investigation of the collagen structure of certain veins. The findings in the case of the thoracic inferior vena cava of the rabbit and of the cat formed so definite an entity that I published them as a preliminary communication in this *Journal*(1). The present paper contains such further details as I have since been able to collect on the collagen structure of other veins, but I have also included in it additional observations, which are of importance in connection with the venous return. One at least of the generally accepted mechanisms of this return is at present the subject of considerable dispute, and I wish, therefore, to do certain further experiments before publishing my own conclusions. The anatomical findings which follow here will be relevant in the light of the physiological experiments which I hope to describe later, and will meanwhile, perhaps, be pardoned for any apparent disconnectedness, for "parmi les connaissances nombreuses que le physiologiste doit posséder, l'anatomie se place sans contredit au premier rang" (Claude Bernard).

In the preliminary communication, referred to above, it was shown that the thoracic inferior venae cavae of the rabbit and of the cat have a tunica media, which is essentially composed of a double criss-cross spiral of collagen fibres, and a tunica adventitia, which is essentially composed of longitudinal elastic fibres. The arrangement of the collagen fibres has since been examined in various veins from laboratory animals, from the slaughterhouse, and from the Zoological Gardens. Dissections have been made with the naked eye, or with the help of a lens, and, where possible, have been corroborated by histological sections. With this technique one can be certain of positive findings, but not always of negative findings. On the whole, however, the results are not opposed to the hypothesis that the collagen fibres of veins tend to be spirally arranged, where these vessels are subject to functional variations in length. I suspect an exception to this rule in a certain type of animal, but I have not yet had enough material to state this with certainty, and in any case it is probable that an adequate physiological explanation can be given for the exception, if it does exist. Since sending the preliminary communication to

press, I have found that Benninghoff (2) deduces the probability of a spiral collagen structure of veins, on the ground of Triepel's observations that veins shorten in length on removal from the body. "Aus dem Vorstehenden," he says, "ist zu schliessen, dass ähnlich wie bei den Arterien auch das Bindegewebe der Venenwand nicht in der Längsrichtung verlaufen kann, denn die kollagenen Fasern müssen so geordnet sein, dass sie die genannten Verlängerungen zulassen. Es kämen Fasernetze in Frage, deren Anteile spiralig verlaufen." As Benninghoff's approach was different from my own, his deductions form a welcome confirmation. As a matter of fact, the possibility of a spiral structure of veins has been in my mind ever since I read Carey's paper on the intestine (3). The hypothesis which I have advanced demands a knowledge of the movements of the veins examined; I have encountered some difficulties of interpretation, in consequence, for lack of a really comprehensive account of the comparative physiology of respiration and of the venous return; these two functional problems present differences, I suspect, in every species, and require for their solution the technique of X-ray cinematography, and a consideration of the normal habits of individual species. The two X-ray photos which are reproduced with this paper illustrate this point. It is my intention to continue research along these lines, if possible, and also to extend the observations on vein structure, but such a programme will be long in fulfilment, and hence this interim communication, in which are presented such data as I have accumulated up to date.

MAN

Superior vena cava. A specimen from a male, aged 21, showed fenestration, but no spiral structure, of its collagen.

Inferior vena cava (*thoracic*). In a specimen from a case of tubercular meningitis the outer part of the vein was composed of dense diaphragmatico-pericardial connective tissue, and neither it nor the inner layer showed any spirality. This was to be expected, if the foramen quadrilaterum does not descend on inspiration, and there is evidence for this view in the radiological findings after phrenic avulsion. Woodburn Morison (4) also states that the middle portion of the central tendon is fixed "by reason of the fibrous portion of the pericardium, which is implanted into it, being connected above with the deep cervical fascia."

Inferior vena cava (*abdominal*). Three specimens were examined. The first, from a male aged 24, had tough connective tissue externally and relatively weak connective tissue internally. No spirality was seen in this or in the next specimen, which came from a male aged 23. The second vein was noticeably narrower below the renal entries. A glass tube was tied in below the renals, and the tributaries were ligatured. Mercury was then poured into the tube, the vein became distended with the first inflow, and thereafter remained without any further apparent distension up to a pressure of 60 cm. Hg. To test this point further, a third specimen was obtained from a male aged 21. The lower end of the vein was clamped off and a glass tube inserted as before. When the

pressure of mercury was increased from "zero" (the top of the vein segment) to 67 cm., the diameter of the vessel was increased only from 20·75 mm. to 21·5 mm. There was a small leak from the side of the vein, where slight damage had been done to the tough outer layer of connective tissue, so it would seem that it is this layer which resists over-distension. The abdominal vena cava, then, has an almost constant maximum volume in the erect position.

Suprarenal vein. There was a possibility, in view of Henderson's findings (5), that a spiral collagen structure might exist in this vein, but three veins which were examined gave negative results.

CAT

Superior vena cava. This showed a double criss-cross spiral of collagen fibres, but it was not so marked as that found in the inferior vena cava (thoracic) of the same species.

External jugular vein. A venous cannula was tied into one such vessel, and the other end of the vein was ligatured. Pressure was then put on the vein with a syringe, and a double criss-cross collagen spiral at once became visible to the naked eye. It was even clearer after dissection, although still not so pronounced as that in the thoracic inferior vena cava. (Histological confirmation of the structure was made on another specimen.) The syringe was then replaced by a glass tube, connected to the venous cannula by a short piece of rubber tubing, the whole was filled with fluid, and the height of this fluid in the tube was used as an index of the variation in volume of the vein. The vessel assumed the position of maximum volume under the slight pressure of fluid present within the tube, and in this position had a length of 20 mm. Shortening its length decreased the volume a very little, stretching it to 27 mm. caused the fluid to rise 16 mm. in the tube, and squeezing out the residual fluid produced a further rise of 1 mm. So stretching decreased the vein volume by $\frac{16}{17}$.

A further point about this vein, which I had often noticed in decapitate preparations, was its great extensibility, so I took the opportunity to make a quantitative measurement on another cat. The length of a selected part of the left external jugular vein in this animal with head retracted (i.e. its maximum normal length) was 34 mm. I cut one end, held the other with artery forceps, and measured the length to which it could be stretched without violence. It was 70 mm. So this vein can stretch to twice its maximum normal length. If left, it will contract slowly back to its original length. To allow this degree of stretching, the collagen fibres, if indistensible, must make an angle of about 60° with the long axis of the vein in the original position, which is theoretically, therefore, near the position of maximum volume (1). So any appreciable lengthening of this vein in experimental work will diminish the lumen, and will render the insertion of a venous cannula more difficult, as one finds.

Inferior vena cava (abdominal). A recent cadaver was put in the supine position, all the branches of the abdominal inferior vena cava were ligatured, and two glass tubes were tied in, one at the hepatic end, the other at the iliac

end. The length between two points in the expiratory position of the diaphragm (as far as one could reconstruct it) was 55 mm. The vein was taken out with its tubes, and its maximum volume was at a length of 62 mm. A diminution of $\frac{1}{38}$ in volume occurred on shortening to 55 mm., and a diminution of $\frac{27}{38}$ on lengthening to 70 mm. The entry of tributaries may have complicated these volume variations a little, and so may have the supine position. When the outer layer of the vein was dissected off, a criss-cross spiral was seen, but it was not so clear as in the thoracic inferior vena cava of this species, and the maximum volume was not at the theoretical angle of intersection of the fibres; this again, however, may have been due to tributary entries. The volume variations were, at any rate, still present after removal of the outer layer. The spirality becomes less pronounced the further caudally it is examined.

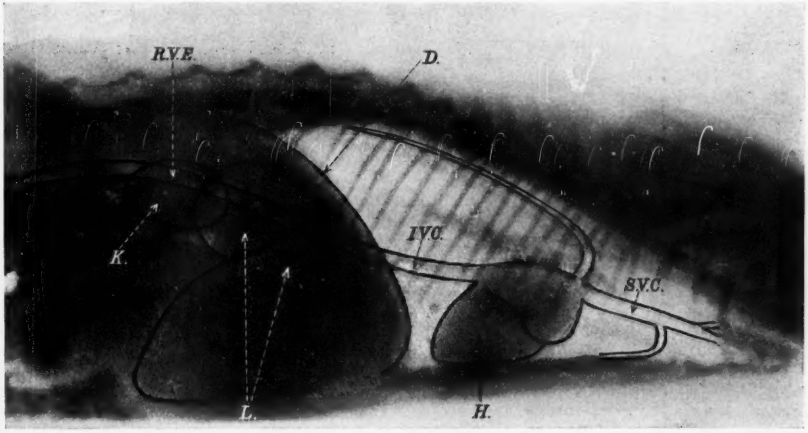

Fig. 1. X-ray photograph of cat. *H.* Heart. *S.V.C.* Superior vena cava. *I.V.C.* Inferior vena cava. *D.* Diaphragm. *L.* Parts of liver. *K.* Kidney. *R.V.E.* Renal vein entry.

The normal lie of the venae cavae. In experimental work on the supine anaesthetised animal, I have noticed that the inferior vena cava passes through the diaphragm very near its middle, and not close to the spine. It occurred to me to see what was the normal situation of the venae cavae, and in this I was helped by the fact that the thoracic inferior vena cava can be seen in an X-ray photograph (6). Dr David Barnes most kindly undertook to photograph a cat for me, and the result is shown in fig. 1. The cat was a female (this sex has the longer thorax) weighing 2600 gm. The only difference from the normal squatting position which I effected was to draw forward a little the fore-limbs and backward a little the hind-limbs. The arch of the back is therefore less emphasised than the normal in the photograph. In the untouched photograph the diaphragm, heart, and thoracic inferior vena cava shadows were quite definite. The remainder of the course of the vein and other details were filled

in as follows. The animal was killed, part of the skin reflected, and the right fore-limb removed between ligatures. The thoracic wall was taken away on the same side above the diaphragm, and the right lung was cut off between ligatures. The carcase was now put in the position it occupied during the X-ray photography, and, by means of accurate measurements from fixed points visible on the photograph, the outlines of additional vessels, etc., within the thorax were added. Then the abdomen was opened, and, with a similar technique and the observation of like precautions, the course of the abdominal vena cava and other details were also entered on the photograph. The result shows that, in the normal squatting position of this animal, the blood has a downward course to the heart from a point caudal to the entries of the renal veins. The cat takes strenuous exercise in short bursts only, and during a large part of its waking, and sometimes of its sleeping, hours it adopts the posture shown in the photograph. The venous return in this animal is therefore very different from that in Man, and supports the statement made above, that this particular functional problem must be studied separately in every species, although allied species will present similarities in accordance with their similarities of habit. For instance, it has been written that "the lion can charge at a speed of about fifty miles an hour over about fifty yards," but "the lion is not a good runner, and will seldom continue a chase once his first speed and energy are exhausted." It is not improbable that in physiological experiments on cats, in which the animals are placed in the supine position, we alter quite appreciably the normal venous return.

RABBIT (DOMESTIC)

Superior vena cava. This is best examined in a doe, as the thorax is longer in that sex, though the vein is short enough even then. Spirality of the collagen fibres was visible without dissection, and stretching the vein decreased its volume, but its elasticity was far less than that of the thoracic inferior vena cava. The spirality became clearer with a little dissection, but it also was less marked than that of the thoracic inferior vena cava. The superior vena cava of the rabbit, lying supine under ether, with the thorax open on one side, shows very little, if any, lengthening on ordinary inspiration, but perhaps in its natural state the partial dependence of the heart from it may cause some slight variation in its length.

Inferior vena cava (abdominal). Owing to the extreme thinness of its wall, this vein could not be examined in the same way as that of the cat; it had to be washed clear of blood, and to have a glass tube passed down inside it. The outer fibres were chiefly longitudinal, and no spirality was visible.

The normal lie of the venae cavae. The last finding made it improbable that there is any great normal variation in length of the abdominal vena cava in the domestic rabbit, and a study of its habits and posture confirms this. It is an inactive animal, and spends most of its time in a squatting position. When it rises off its fore-limbs, it remains so for only a short time. When it runs it tends

to rise further on its hind- than on its fore-limbs. Dr David Barnes kindly took for me an X-ray photograph of a young rabbit in its normal position (fig. 2). This showed the thoracic inferior vena cava, heart, superior vena cava, and diaphragm. The rest of the inferior caval course, etc., I drew in by means of a technique similar to that which I used for the cat. It will be seen that in this animal the heart is at an extremely low level, and that the inferior vena cava slopes sharply downwards from a point somewhat caudal to the entries of the renal veins. Incidentally, the difference in size of the thoracic cavity in the cat and in the rabbit is very noticeable, and is doubtless to be correlated with the great difference one finds in the respiration rates of these animals.

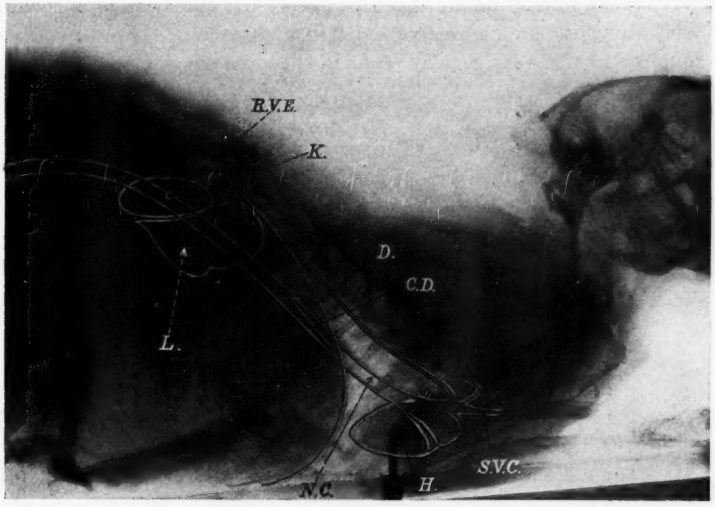

Fig. 2. X-ray photograph of rabbit. *H.* Heart. *S.V.C.* Superior vena cava. *N.C.* Inferior vena cava. *D.* Diaphragm. *C.D.* Centre of diaphragm. *L.* Part of liver. *K.* Kidney. *R.V.E.* Renal vein entry.

DOG

Superior vena cava. Accurate measurements kindly made for me by Dr H. P. Gilding show that this vein does not alter in length in inspiration. Corresponding with this, I found no definite spirality of the collagen fibres. The adventitia is very tough.

Inferior vena cava (*thoracic*). Horizontal and longitudinal sections, stained with haematoxylin and van Gieson, and with picro-indigo-carmine and orcein, show that this vein contains few, if any, muscle fibres except at the level of the diaphragm. It is, therefore, similar in this respect to the corresponding vein in the cat and in the rabbit. The vein lengthens on inspiration (H. P. Gilding) to about $\frac{9}{8}$ its expiratory length. It has volume variations according to its length, but the position of maximum volume varies with the individual dog. It has

an elastic recoil after stretching. Under the adventitia is a double criss-cross spiral of collagen fibres: the intersection of the spirals is more acute on one aspect (anterior?) than on the other (fig. 3). Stereoscopic photographs show that one spiral is external to the other, and that there is no interlacing of the two sets of fibres. It seems possible that the more acute intersection of the spirals on one aspect may be due to a greater caudal movement of the diaphragm on inspiration on the opposite aspect, but I have been unable to get information on this point.

SHEEP

Superior vena cava and azygos vein. On removal of the outer layer from these vessels a double spiral arrangement of collagen fibres was at once visible. That in the azygos vein, before fixation, had an appearance like the scaly bark of a conifer and was very regular. That in the vena cava was coarse and not very regular.

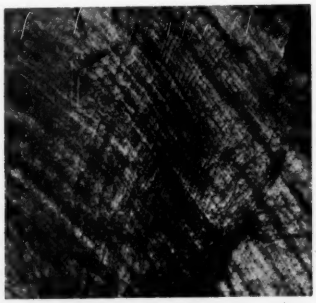

Fig. 3. Photograph of double criss-cross spiral of collagen fibres in the inferior vena cava (thoracic) of a dog.

Inferior vena cava (thoracic). I dissected the adventitia off from one specimen, and found that the underlying fibres were chiefly longitudinal, with very little criss-crossing. From a second specimen I removed both adventitia and intima with a similar result. Both these veins had a maximum volume at a particular length, but they allowed far less stretching than I had expected. It is possible that in this animal the superior vena cava is the more movable vein, and that the base of the heart moves caudally when the diaphragm contracts. Other possibilities are that the angle of Lower becomes less acute on inspiration, or that the caval orifice in the diaphragm retains its position in inspiration, but the solution must wait upon X-ray examination.

HYBRID PERSIAN AND MARICA GAZELLE

Neither vena cava from the thoracic cavity showed spirality of its collagen fibres.

SAMBUR DEER

Superior vena cava. Length 125 mm. No spirality was seen.

Inferior vena cava (*thoracic*). Length 130 mm. The adventitia was very strong and probably acted as a resistance to distension. The collagen of the media showed a definite double criss-cross spiral, which resembled that of the dog in having a more acute angle of intersection on one aspect than on the other.

RING-TAILED LEMUR

Both the superior vena cava (length 35 mm.) and the thoracic inferior vena cava (length 30 mm.) showed very regular criss-cross spirals of collagen fibres, that in the latter being more pronounced than that in the former vein.

NOTE ON THE PRESERVATION OF SPECIMENS

Some fixatives, such as formol, are unsuitable for this purpose. At Mr W. Chesterman's suggestion I tried 0·1 per cent. chromic acid, and Zenker's solution. After the material has been in either solution for a day, it can be transferred to 70 per cent. alcohol, and seems to keep well.

CONCLUSION

A spiral arrangement of collagen fibres is described in certain further veins, and a working hypothesis is advanced to account for such spirality. Some other points of interest are noted about certain veins in connection with the venous return.

I wish to thank Dr H. P. Gilding, Mr T. H. Sellors, Dr S. Zuckerman, Mr D. R. Balcombe-Brown and Mr J. W. Litchfield for supplying me with some of the material for dissection, and for the data accompanying this supply. To Dr David Barnes I am indebted for the X-ray photographs. Mr Marsland, of the University Department of Physiology, Oxford, kindly prepared the histological sections, and Mr W. Chesterman, of the University Department of Human Anatomy, Oxford, photographed the vena cava of the dog. I should like, finally, to acknowledge the help of our own laboratory staff in these investigations.

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THE VASCULARITY OF THE VALVES OF THE HUMAN HEART[1]

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Luschka (23, 24) was the first to demonstrate that both the atrio-ventricular and semi-lunar valves contain blood vessels, giving a detailed description of his methods of investigation, and the results which he obtained. He was subsequently corroborated in his results by Rosenstein (30), Krause (18), Kolliker (14, 15) and Forster (7).

Rokitansky (29), Joseph (13), Virchow (33) and Cadiat (4) were unable, however, to verify these findings.

On the other hand Gerlach (9), Frey (8), Sappey (31), Henle (12) and Cöen (5) have all demonstrated that normal atrio-ventricular valves are vascular, but that no blood vessels exist in the semi-lunar valves.

Langer (21) maintained that blood vessels are found only in valves which contain muscle fibres.

Darier (6) after extensive investigation found that blood vessels never exist in the fibro-elastic parts of the atrio-ventricular valves except in the case of the aortic cusp of the mitral valve into which he observed vessels extending for not more than one-sixth of its length. He noted the presence of vessels in all valves which were diseased, and maintained that those investigators who had succeeded in injecting human valves had been dealing with pathological specimens.

Confirmation of the work of Langer and Darier came from Odinzow (27) in 1904 and Koniger (16).

In 1912 Nussbaum (26) described vessels in the atrio-ventricular valves which extended for only 3 mm. from the line of attachment of the cusps to the wall of the heart, but was unable to show by injection any blood vessels in the pulmonary or aortic valves. The question of the vascularity of the valves of the heart was reopened in 1917 by Bayne-Jones (2) who injected fourteen human hearts and obtained a fairly complete injection of both atrio-ventricular and semi-lunar valves in three of his specimens. His observations and illustrations (reproduced in Maximow's Histology (25)) showed that a vascular network pervades practically the whole extent of the atrio-ventricular valves. He attributed to faulty technique the failure of other workers to make a complete injection of the vessels in these valves.

In 1921 Gross (10) reported that he had succeeded in injecting the mitral

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valve in 6 per cent. of normal hearts representing all age periods from birth to the ninth decade, and was of opinion that the presence of vessels in the valves had some relation to the age and persistence of muscle tissue in them.

At that time he was unable to give accurate statistics on his own observations of the frequency with which valves could be injected at different age periods.

Kugel and Gross (19) after an examination of eighty-five human hearts observed that seven showed "the complete form of vasculature," but although all these showed a co-existing rheumatic valvulitis they were unable to depart from the conclusion that the larger vessels were not of inflammatory origin.

Kugel (20) in 1928 gave a detailed description of a vessel in the interatrial septum, which he named the "arteria anastomotica auricularis magna," previously observed by Kugel and Gross (*loc. cit.*), which may send branches to the aortic cusp of the mitral valve and to the aortic valve. Kugel did not, however, find these branches constantly present, but considered that they played an important part in the pathogenesis of valvular endocarditis.

Ritter, Gross and Kugel (28) after a further study of six hundred and fifteen additional human hearts found fourteen cases with blood vessels in "some of the valves." A thorough clinical and pathological examination failed to reveal that these vessels owed their origin to inflammation.

It has occurred to investigators that the key to the solution of the problem of vascularity might be found by studying the muscle content of the valves, but on reviewing the literature, we find that the reports which deal with the muscle content of valves are almost as varied as those dealing with the presence or absence of the blood vessels in them. Numerous conflicting accounts appear concerning its extent in the valves at different age periods, and of its source from atrium and ventricle. The presence of atrial muscle in the atrio-ventricular cusps is generally admitted, but Langer (22), Darier (*loc. cit.*), Odinzow (*loc. cit.*) and Tandler (32) found that it is of greater extent in children, while Albrecht (1) reported that it is better developed in the adult.

Langer (22) further noted that the aortic cusp of the mitral valve contained most muscle and was the last to lose it, but the order of disappearance of muscle in other cusps has been variously stated.

Walmsley (34) in Quain's *Anatomy* (p. 45) considers that the auricular muscle fibres "do not extend beyond the upper third of the cusp, but end below in pointed processes on the atrial surface of the lamina fibrosa." The presence of ventricular muscle in the cusps has been described by Bernays (3), Langer (21) and Henle (12).

It is thus evident that for many years the vascularity of the valves of the heart has been the subject of investigation. One can readily appreciate the importance of this work, for the presence of blood vessels in valves is essential to the theory of embolic valvular endocarditis. With the conflicting evidence both as regards blood vessels and muscle content it is not surprising that some maintained that blood vessels are present in human valves only when these are

the seat of inflammation, and others that a small percentage of normal valves show a definite and well-organised system of blood vessels.

Consequently as regards the production of valvular endocarditis two main views exist, the one represented by Köster (17) that normal valves are vascular and that bacterial emboli enter the valve by these vessels and become arrested at the closing edge of the valve, the other denying the presence of blood vessels in human normal valves, and being of the opinion that valvular endocarditis is caused by bacteria deposited on the valves from the main blood stream.

MATERIAL AND TECHNIQUE

The material on which we report in this paper consisted of forty human hearts, collected from the post-mortem room of the Dundee Royal Infirmary.

They varied in age from 1 day to 82 years, ten of them being under 1 year, and seven between 1 and 5 years. Three specimens showed definite valvular endocarditis, but the others were normal to inspection, and without a clinical history of heart trouble.

The method of examination was as follows: The hearts were kept until rigor mortis had passed off. Cannulae were then inserted into the orifices of the coronary arteries in the sinuses of Valsalva, a ligature being tied round the proximal part of each vessel. All exits from the chambers of the heart except the inferior vena cava and coronary sinus were tied, and the heart perfused with warm normal saline for at least 10 min. or until the washings were perfectly clear.

The inferior vena cava and coronary sinus were then tied and the injection made at 150–200 mm. of mercury. The coronary arteries of the smaller hearts were injected by inserting and tying a cannula into the ascending aorta.

Various fluids were used for injection, Berlin blue, Gelatin carmine, Mandarin pen black (Indian ink) and ethereal lard. We found a fine gelatin carmine mass (Ranvier) and a 3 per cent. aqueous solution of Leichtlosl Berlin blue 1*a*, among the most suitable.

Microscopic examinations of the injection masses were made prior to injection to make sure that they did not contain particles.

The hearts were fixed immediately after injection in 10 per cent. formalin, and valves from different age periods were sectioned, the paraffin method being used for the most part. The sections were examined microscopically specifically for muscle, and the stain used was van Gieson. Several of our specimens were cleared by the Spalteholz method and permanently preserved in three parts methyl salicylate to one part benzyl benzoate, a method recommended by Wilson (35) for thick serial sections of embryos.

RESULTS

(*a*) *Normal hearts*

In none of the thirty-seven normal hearts have we observed a blood vascular network throughout the whole extent of any valve or cusp. On detailed examination of seventeen hearts of the first decade seven showed the injection mass extending into the vessels of the atrio-ventricular valves for a very short distance only, but none in the semi-lunar valves. In two of those where it was most marked the extent of this injection was not more than 3 mm. from the line of attachment of the cusps to the wall of the heart (fig. 1) while in the others it was much less. In these seven cases, vessels were observed both in the tricuspid and mitral valves (see Table).

Microscopic examination revealed that these vascular extensions into the valve segments were associated with the presence of auricular muscle in the cusp, and the vessels of the muscle extensions were directly continuous with those of the adjacent auricular musculature (fig. 1). Further, the distal portion of these vascular extensions was observed to be made up of completely filled terminal capillary loops (fig. 3), so that there seems little doubt that the injection obtained was complete.

We have sectioned the aortic cusp of the mitral valve and the septal and infundibular cusps of the tricuspid valve specifically for muscle content, and found that in them muscle extension was as frequent in the cusps on the right as on the left side of the heart and that it extended downwards only for a short distance.

The examination of the twenty hearts of our series which were over 10 years of age showed that it was possible to inject the aortic cusp of the mitral valve more frequently than in any of the other cusps, and further the extent of the injection was never more than 2 mm. from the base of the cusp. We injected the aortic cusp of the mitral valve in seven cases and the septal cusp of the tricuspid in three.

As in those of the first decade no injection was obtained in either the aortic or pulmonary semi-lunar valves nor have we observed any vessels passing from papillary muscles along chordae-tendineae towards the valves. Islands of muscle in the cusps described in the literature do not appear to be common, strands of muscle fibres may appear insular in single sections, but by examining serial sections, continuity with auricular muscle can usually be demonstrated.

Bayne-Jones (*loc. cit.*) states that the inability of other observers to inject heart valves is due to a faulty technique, but we consider our demonstration of completely filled capillary loops (see fig. 3) in normal valves is conclusive that we have succeeded in demonstrating their complete vascularity. In addition we have been successful in injecting capillary networks in our cases of endocarditis, and have demonstrated capillaries in the normal valves of the pig and sheep where they extended throughout a considerable part of the valve.

The following table gives details of seven hearts of the first decade which showed the injection mass extending into the atrio-ventricular valves.

P = pressure at which injection was made.
I.M. = injection mass which was used.

1. Female foetus aet. 8½ months. Lived 2 weeks. Cause of death meleana neonatorum, I.M. Gelatin carmine. P. 100 mm. Hg.	Septal cusp of tricuspid. Aortic cusp of mitral (slight).
2. Female aet. 1 day. Cause of death broncho-pneumonia. I.M. Berlin blue. P. 100–5 mm. Hg.	Septal cusp of tricuspid.
3. Male aet. 2 days. Cause of death congenital absence of abdominal wall. I.M. Gelatin carmine. P. 100 mm. Hg.	Septal, infundibular, and marginal (slight) cusps of tricuspid. Aortic cusp of mitral.
4. Male aet. 2 weeks. Cause of death broncho-pneumonia. I.M. Berlin blue. P. 120 mm. Hg.	Aortic and posterior (slight) of mitral. Septal cusp of tricuspid.
5. Female aet. 6 months. Cause of death broncho-pneumonia. I.M. Berlin blue. P. 100–120 mm. Hg.	Posterior cusp of mitral (slight). Septal cusp of tricuspid.
6. Male aet. 3 years. Cause of death chronic nephritis. I.M. Berlin blue. P. 100–120 mm. Hg.	Septal, infundibular, and marginal (slight) cusps of tricuspid.
7. Male aet. 4⅓ years. Cause of death encephalitis lethargica. I.M. Gelatin carmine. P. 100–120 mm. Hg.	Posterior cusp of mitral. Infundibular cusp of tricuspid.

(*b*) *Hearts with valvular endocarditis*

We have injected three cases of endocarditis affecting the aortic cusp of the mitral valve, and found in them a fairly complete vasculature which extended from the base of the cusp to the line of closure, several arterioles of larger size being observed. These vessels were most clearly seen from the auricular aspect of the cusp and could be traced up into the larger vessels in the auricular musculature. Branches of these arterioles anastomose freely in the cusp and with the vessels in the adjacent auricular muscle (fig. 5). These blood vessels were unassociated with muscle fibres.

The distal portion of the cusp from the line of closure to the free edge was, however, quite avascular except where the vegetations encroached on this area (fig. 5, *B*). In one case, that of a male aged 27 years, the mitral infection was, as is often the case, accompanied by an aortic lesion, where vascularity was again demonstrated. Blood vessels extending from papillary muscles along chordae-tendineae into the infected cusp were observed in all three cases.

The specimen which showed tricuspid infection (fig. 6) in a female aged 52 years, is interesting. Coincident with a chronic mitral lesion is a rich vascularity along practically the whole extent of the thickened free edge of the tricuspid valve. This thickened area probably represents the actual line of closure in this somewhat shrunken valve. The vessels supplying it are derived from two sources, viz. (1) by an arteriole from one of the larger vessels in the

auricular musculature (fig. 6, *B*); (2) by vessels which reached it by passing upwards from a papillary muscle. In addition the septal cusp is supplied at its base by irregular leashes of vessels extending downwards for about 5 mm. towards the free edge. On sectioning a portion of this cusp these vessels were observed to be directly continuous with the vasculature of the adjacent auricular muscle but were themselves unassociated with muscle fibres. It seems unlikely that these vessels had remained in the fibro-elastic substance of this cusp for over 50 years. They had not reached the seat of inflammation in the valve, and it is most likely that they are of the nature of a new formation. Can the same be said of the larger arterioles and their final ramifications in this cusp? Have these persisted from infancy and were they the pathways through which bacteria gained access to this valve? We were unable to ascertain whether or not the endocardium of this valve was wholly intact, but in our opinion the vascularity observed in this case and in the valves of our other pathological specimens is to be regarded as a new growth of vessels.

SUMMARY

1. From an examination of forty human hearts normal to inspection and without a clinical history of heart disease, blood vessels were found in the atrio-ventricular valves, but they did not extend into them for more than 3 mm. from the line of attachment of the valve to the heart wall.

2. Vessels were more frequently present in the tricuspid and mitral valves of hearts under 10 years of age than in those past this decade, and were always associated with an extension of auricular muscle into the cusp.

3. Islands of muscle in cusps are not common and we did not find muscle in them continuous with chordae-tendineae as stated by Albrecht (*loc. cit.*), nor did we observe transverse muscle fibres in them as described by Gussenbauer (11).

4. Blood vessels observed in three cases of endocarditis are regarded by us as of the nature of a new formation.

5. From an examination of the cases at our disposal we do not consider that persistence of muscle in the valves of adult hearts plays any part in the development of embolic endocarditis, for the muscle does not extend into the cusp so far as the line of closure of the valves, i.e. to the part where valvular endocarditis begins.

Our thanks are due to Dr G. R. Tudhope, Lecturer in the Pathology Department, University College, Dundee, for his kindness in supplying us with material.

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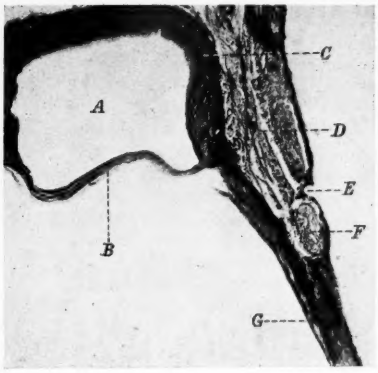
EXPLANATION OF PLATE I

Fig. 1. Vertical section of the aortic cusp of the mitral valve and the semi-lunar cusp of the aortic valve of a day-old child. The auricular muscle extends into the aortic cusp of the mitral valve for approximately 1·5 mm. Injected with a 2 per cent. aqueous solution of Mandarin pen black. Stained with van Gieson. Muscle yellow. Fibro-elastic part of valve red. *A*, sinus of Valsalva. *B*, semi-lunar valve. *C*, wall of aorta. *D*, auricular muscle. *E*, base of cusp (line of attachment). *F*, muscle extension into valve (injected). *G*, fibro-elastic avascular portion of the valve.

Fig. 2. Vertical section of the aortic cusp of the mitral valve of a child 2 years 11 months old. The auricular muscle extends only to the base of the cusp. Injection as in fig. 1. *A*, semi-lunar valve (left coronary cusp). *B*, auricular muscle (injected). *C*, base of the cusp. *D*, fibro-elastic avascular portion of the valve.



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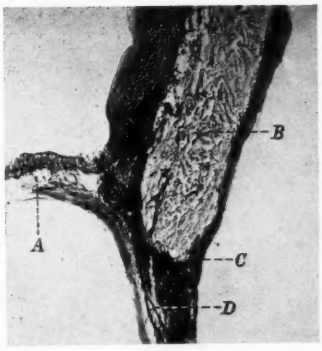

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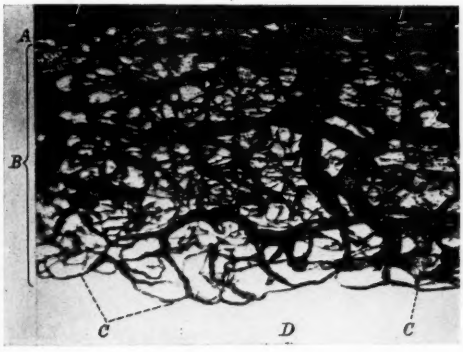
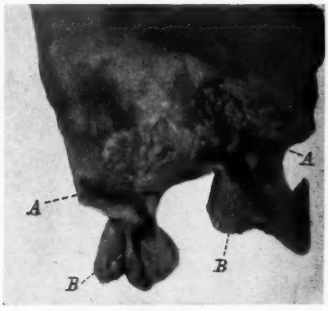


Fig. 3.



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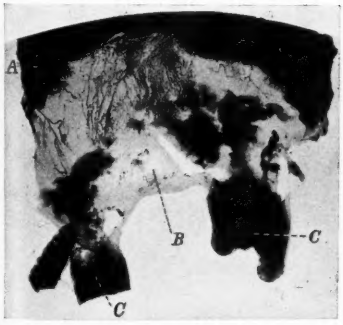

Fig. 5.

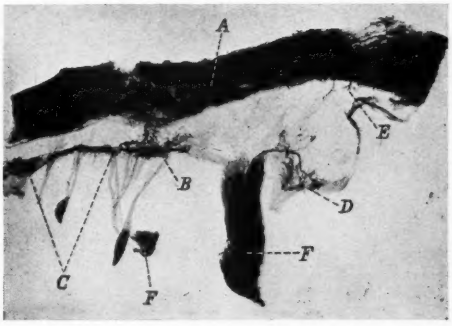

Fig. 6.

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Fig. 3. The base of the aortic cusp of the mitral valve of a day-old child, injected with 3 per cent. aqueous solution of Indian ink, and cleared. Capillary loops are seen extending into it for approximately 1 mm. *B*, from the line of attachment *A*, of the cusp to the heart wall. *C*, terminal capillary loops. *D*, fibro-elastic avascular portion of the valve.

Fig. 4. Specimen of endocarditis of the aortic cusp of the mitral valve in a male 27 years of age. Vegetations *A* are seen on the auricular aspect. Injected with 3 per cent. aqueous solution Berlin blue. *B*, papillary muscle.

Fig. 5. Same specimen as in fig. 4. Cleared. Vessels are seen passing downwards from the base of the valve towards the vegetations. *A*, auricular muscle. *B*, avascular portion of the valves. *C*, papillary muscle.

Fig. 6. Specimen of endocarditis of the tricuspid valve in a female aged 52 years. Injected with 3 per cent. aqueous solution of Berlin blue and cleared. Vessels are seen passing downwards from the base of the valve and upwards from the papillary muscle. *A*, auricular muscle. *B*, arterioles passing from the base of the valve to the thickened free margin *C*, of the infundibular cusp. *D*, thickened free margin of the septal cusp with large arteriole passing up from papillary muscle *F*. New formation of blood vessels *E*.

A NOTE ON THE OCCURRENCE AND NATURE OF THE PIGMENT IN THE PARS NERVOSA OF THE HUMAN HYPOPHYSIS

By RUBY O. STERN

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Histological descriptions of the posterior lobe of the human pituitary body invariably include the statement that brown pigment granules are sometimes to be found amongst the neuroglial fibres, but little information is available regarding the nature of these granules and their occurrence in normal and pathological conditions.

Cooper, who examined a large number of pituitaries from patients of various ages, did not express any opinion on the nature of the pigment, but appeared to agree with Lim who considered this pigment to be identical with that found in the zona reticularis of the suprarenal cortex. She mentioned the fact, however, that she had observed large masses of pigment in the interglial spaces of the pituitary from a young woman aged twenty years.

Other writers, notably Blair Bell, Köhn, Fischer and Biedl, have subscribed to the view that this pigment is a degeneration product which occurs in increasing amount with advancing age. Köhn observed tiny quantities of pigment in the pituitary of a child of four years and very large amounts in the pituitaries of subjects between the ages of sixty and seventy years. Pende considered the pigment to be the product of a secretory activity of the primitive glial cells in the posterior lobe. Livon and Peyron also regarded the pigment as resulting from activity of the cells in the posterior lobe, though they postulated a process of elaboration of the products of the anterior lobe which had passed through into the posterior lobe rather than a specific secretion of the cells in the posterior lobe. Stumf did not recognise any connection between the glial constituents of the posterior lobe and the pigment, since he observed it not only between the tissue spaces in the posterior lobe but also in the anterior lobe.

Some divergence of opinion has also been expressed as to the precise position of the pigment deposits in the posterior lobe. Blair Bell found it only in the most posterior part of the lobe; Köhn observed most pigment in the anterior and middle parts; Cooper found pigment present only in the most anterior part adjoining the pars intermedia. Whereas Köhn described the pigment as occurring mainly in the neuroglial cells and along their processes, Cooper found it only in clumps which lay in the tissue spaces outlined by neuroglial fibres.

The histo-chemistry of the pigment has been investigated by Köhn and by Clunet and Jonnesco, who were alike unable definitely to determine its chemical constitution. Köhn stated only that he considered the pigment to be neither a fat nor a lipochrome such as occurs in the cells of the nervous system in advanced age. Clunet and Jonnesco, who submitted the pigment to a large number of chemical tests, came to the conclusion that it was allied to the melanin group. Lim, who maintained that this pigment in the pituitary was identical with that in the zona reticularis of the suprarenal, described the latter only as a non-lipoid, iron-free pigment.

Through the kindness of Dr J. Godwin Greenfield, who removed the pituitaries for me at post mortem, I have been enabled to study the pigment in the pars nervosa in over a hundred cases of diseases of the nervous system, some cerebral, some purely spinal. The specimens were taken at random and from patients of all ages. The pigment was investigated from three aspects: (*a*) its chemical nature, (*b*) age incidence, and (*c*) distribution of the pigment in the gland.

The majority of pituitaries were fixed in Bouin's fluid; sagittal sections were taken through the stalks, and subsequently sections were cut in paraffin. A few were fixed in formol saline, prior to being cut by the frozen method. The routine stains were a modification of Mallory's aniline blue orange G. stain and iron-haematoxylin with an aqueous eosin counterstain. Special stains were used for histo-chemical examination.

CHEMICAL NATURE OF THE PIGMENT

The fact that the pigment was visible in paraffin sections was sufficient evidence that it was insoluble in the ordinary fat solvents, but to exclude the possibility of its being a lipoid both paraffin and frozen sections were immersed for twenty-four hours in acidified absolute alcohol. At the end of that time the pigment persisted as golden brown granules when stained by the routine methods. In addition the pigment did not give the orange-red colour of lipochrome when stained with Scharlach R. either before or after immersion in acidified alcohol. This failure to stain with Scharlach R. differentiated the pigment from that in the zona reticularis of the suprarenal which gave the typical colour of lipochrome with this dye after the other lipoids had been dissolved by treatment with acidified alcohol for twenty-four hours. It is not possible therefore to agree with Lim that these two pigments are identical. Further proof of the difference between them was furnished by their behaviour to Masson's ammoniacal silver stain for melanin. I obtained an indication that the pigment in the pituitary might be melanin from the observation that after treatment with hydrogen peroxide for twenty-four hours many pigment granules were dissolved. By Masson's method the pigment in the pituitary stained an intense black; that in the suprarenal remained brown as seen in sections stained by routine methods. Control sections of a melanoma were made at the same time and the melanin pigment in these stained similarly

to the pigment granules in the pituitary. The chemical composition of these granules therefore appeared to be that of melanin.

AGE INCIDENCE

The first hundred pituitaries examined were divided into four age groups, which corresponded roughly to the periods of growth (birth to twenty years), of maturity (twenty to forty-five years), of involution (forty-five to sixty years), and of senility (over sixty years). The amount of pigment present was noted as *A*, none, *B*, a few granules only, *C*, a fair amount, *D*, a large amount, and *E*, a very large amount. It was seen at once from a consideration of the total number of cases in which pigment was present that large amounts occurred only exceptionally. In 39 per cent. of cases no pigment was present; and in another 34 per cent. only a few granules were present, giving a figure of 73 per cent. of cases in which pigment was practically absent. Fairly large amounts were found in 10 per cent. of cases, large amounts in 11 per cent., and very large amounts in only 6 per cent.

The youngest age at which pigment was seen was seven years, a few granules only being visible. But in the pituitary of a child aged twelve years there were a fairly large number of granules. The following table gives the number of pituitaries examined in each age group; the relative amounts of pigment present in each group are indicated by letter.

The Relative Amounts of Pigment Present at Various Ages

Age of patient	No. of cases	No. in pigment groups
Birth to 20 yr.	18	(*A*) 14, (*B*) 3, (*C*) 1, (*D*) 0, (*E*) 0
20–45 yr.	38	(*A*) 12, (*B*) 15, (*C*) 5, (*D*) 5, (*E*) 1
45–60 yr.	36	(*A*) 11, (*B*) 11, (*C*) 4, (*D*) 5, (*E*) 5
Over 60 yr.	8	(*A*) 2, (*B*) 5, (*C*) 0, (*D*) 1, (*E*) 0

(*A* = no pigment, *B* = a few granules only, *C* = a fair amount, *D* = a large amount, *E* = a very large amount.)

From the table it can be seen that the largest amounts of pigment were found in the age group forty-five to sixty years. But whilst pigment was certainly absent in a very high proportion of cases during the period of growth (in about 77 per cent.), it was also absent in 25 per cent. of cases over the age of sixty, and in this group no very large amounts were found. It is difficult therefore to state definitely that the pigment is a degeneration product which occurs with advancing age. If this were so the pigment should have been present in very large amounts in cases over sixty years of age.

There was no relationship between the disease from which the patient suffered and the amount of pigment present. A special note was made of those cases in which the pituitary had been compressed either directly or indirectly by intracranial neoplasms and in which the cells of the posterior lobe might conceivably have been damaged with consequent formation of products of degeneration. But no such relationship could be determined.

DISTRIBUTION OF THE PIGMENT IN THE GLAND

In the majority of cases the pigment appeared in clumps consisting of rather large granules which stained a yellowish brown colour with iron-haematoxylin and a golden brown with the orange G. component of the aniline blue orange G. stain. There was little predilection of position in the gland: the pigment lay scattered indiscriminately throughout the posterior lobe although the largest clumps were seen in the middle of the lobe. A few granules were occasionally present in the neuroglial cells but the largest accumulations of pigment were between the neuroglial fibres in the tissue spaces. In several cases pigment granules were visible in the stalk of the pituitary where they lay free in the tissue spaces. No pigment was seen in the anterior lobe. Though not strictly relevant it may be of interest to record that only in one pituitary did I find any of the hyaline bodies which Herring described and in this one only a few were present.

SUMMARY

1. The chemical reactions of the pigment in the posterior lobe of the pituitary are identical with those of melanin. In this respect the pigment differs from the pigment in the zona reticularis of the suprarenal cortex, which gives the reactions of a lipochrome.

2. An appreciable amount of pigment occurs only in about 27 per cent. of pituitaries. It occurs in greatest amount from the fourth to the sixth decade of life though it may be present as early as the seventh year and absent as late as the sixty-eighth.

3. There is no definite evidence that the pigment is the product of cellular degeneration.

4. The pigment may occur in any part of the posterior lobe as well as in the stalk of the pituitary though the largest accumulations of pigment are situated between the neuroglial fibres in the middle of the posterior lobe.

I am grateful to Dr J. Godwin Greenfield for his help in enabling me to obtain material for this study.

The work was carried out during the tenure of a part time grant from the Medical Research Council for which I desire to express my thanks.

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NERVE ENDINGS IN HUMAN TEETH

By O. W. TIEGS

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The controversy over the termination of sensory nerves in teeth has been concerned mainly with two opposed theories: (i) that the fine nerve fibres enter the dentinal tubes and terminate near the enamel, (ii) that the nerves end in association with the odontoblasts.

The supporters of the former view are especially impressed with the extreme sensitiveness of exposed dentine to stimuli such as slight touch or contact with certain non-volatile chemical substances, i.e. stimuli which cannot owe their effects to mechanical pressure on the underlying pulp or to sudden application of cold to it.

A histological basis for the theory was forthcoming in the work of Boll (1868), who believed he could demonstrate in material prepared with chromic acid a system of nerve fibres entering the dentinal tubes. In the light of present knowledge Boll's method must be regarded as inadequate. Römer (1899) and more recently Dependorf (1913) and Fritsch (1914) have also described an innervation of dentine. The extreme view is expressed in the work of Morgenstern (1896) who believed he could detect in Golgi preparations a dense meshwork of nerve fibres traversing the dentine in all directions, and with nerve cells lodged in the dentinal tubes. The most elaborate recent work in which similar conclusions have been reached is that of the late J. H. Mummery (1919). By a special gold method he found it possible to demonstrate fine fibres entering the dentinal tubules from the pulp, and accompanying the dentinal fibres (Tomes' fibres) throughout the dentine. He believed he could show the origin of these fine fibres from nerve cells lying just below the odontoblast layer; and that the coarse medullated nerves from the pulp, approaching the odontoblast layer, broke up into a brushwork of fibrils which formed synapses with these nerve cells.

In a recent re-examination of this work Stewart (1927) finds that Mummery's fibres do not disappear if the inferior dental nerve be cut and allowed to degenerate. Of course if they are the axons of peripheral nerve cells, located as Mummery thought in the pulp, degeneration should not occur. Stewart, however, fails to find any connection between these fibres and supposed nerve cells in the pulp; and it would certainly seem that, if the axons stained in such large numbers, their cell bodies, were they present, would not remain entirely uncoloured. He concludes that unless nerve fibres have reached the teeth via the inferior dental artery, Mummery's fibres cannot be nerves.

The belief that the pulp nerves end in the odontoblast layer originated with Retzius (1892, 1893, 1894) by the use of Golgi preparations of teeth from young fishes, amphibians and reptiles, as well as from a young mouse. Huber (1898) confirmed Retzius' conclusions in methylene blue preparations of rabbit teeth. Retzius and Huber both thought that the nerves ended free among the odontoblasts. Hopewell-Smith (1918), who devoted much time to the matter, concluded that the nerves "terminate in a basket-work of varicose fibres embracing and often closely attached to the cell walls of the individual odontoblasts."

If the nerves end only in association with the odontoblasts, then it is to these cells that the sensitiveness of dentine must be ascribed. The apparent sensitiveness of the enamel may find an explanation in the recent work of Loher (1929) and Allen (1930) who have been able to trace the dentinal processes beyond the limits of the dentine into the enamel. The mesenchymal rather than epiblastic origin of the odontoblasts has been urged as an objection to their serving as pain receptors. But there is no *a priori* reason why sense cells should be exclusively epiblastic; they are predominantly so because it is this layer that is most likely to come in contact with the outer world.

MATERIAL AND OBSERVATIONS

The following observations were made on the molar teeth of a young adult[1]. Immediately after extraction the teeth were put in 10 per cent. formalin, and after hardening several months cut with a carborundum saw into longitudinal slices about 2 mm. thick. The pieces were then decalcified for about a year in a 10 per cent. formalin solution acidified with $\frac{1}{2}$ per cent. hydrochloric acid. When the dentine had become thoroughly pliable the material was transferred to pyridine for two days, and then stained by the Bielschowsky block method. For examination the tissue was embedded in paraffin and cut into thick sections (25μ).

The Bielschowsky method, as is well known, is unaccountably capricious, and many failures or only indifferent impregnations are often obtained; but the extraordinarily brilliant preparations that it sometimes yields compensate for this. The following description is based on the examination of material in which the impregnation had been very successful.

Before proceeding to describe the innervation of the pulp, brief reference may be made to the structure of the region specially concerned, namely the superficial layers of the pulp and the adjoining inner margin of the dentine. In Bielschowsky preparations of decalcified teeth most of the dentine colours only weakly, so that any stained nerve filaments that occurred in it could hardly escape detection. The inner margin of the dentine is very irregular and unfortunately is apt to stain very deeply (see figures 2, 4, 5). Immediately internal to this, in teeth that are not yet fully grown, is the odontogenic zone imperfectly or not at all calcified, but with dentinal tubes already visible.

[1] For the material I am indebted to Dr C. E. Allen of the Australian College of Dentistry, at whose request this work was undertaken.

Beneath this is a space (lymph space?) traversed by the processes of the odontoblast cells (Tomes' fibres), on their way to the dentinal tubes. This subdentinal zone of odontoblast processes is bounded on its inner margin by a peculiar membrane composed of collar-like attachments to the odontoblast processes, and first described by Paul (1902). The existence of these "collars" has been doubted; in the Bielschowsky preparations they appear, however, with great clearness, and seem to be developed from the odontoblasts themselves. The odontoblast cells lie immediately under this "membrane." They are smaller and closer packed in the lower part of the tooth and usually bigger and more loosely packed in the crown. From their inner aspect there arise numerous fine filaments that grow deeper forming a distinct pale layer in the pulp, the layer of Weil. The existence of this zone has been disputed (v. Ebner, Röse) on the ground that it is a shrinkage artefact. But in Bielschowsky preparations, which are admirably adapted for the purpose, its composition from fibres, derived largely from the odontoblast processes, is very clear. It is not equally prominent everywhere; sometimes it is seen to contain branching connective tissue cells (fig. 1).

The nerves, entering the tooth through the apical opening, traverse the pulp mainly in bundles as described by Huber. Many of the fibres are obviously medullated; the Bielschowsky method is not adapted for distinguishing the finer medullated fibres from the unmedullated ones. In the pulp they branch considerably. Just internal to the odontoblast layer the branching is often especially evident (fig. 1), numerous fine filaments thus arising, nonmedullated and often of extreme tenuity. They are frequently varicose and are often hard to distinguish from the fine fibrous processes that grow from the odontoblast cells into the pulp, forming the pale layer of Weil. In thick sections it is possible to trace many of the fine nerve fibres past the layer of the odontoblast cells, almost to the inner margin of the dentine (sub-dentinal zone) where they usually turn and branch, running often for considerable distances parallel to the dentine as excessively fine filaments amongst the odontoblast processes. There are places in the preparations where the odontoblast processes are packed close together and soon enter the dentinal tubes; this is especially the case in the crown region, and precise observations are then very difficult to make. But in those regions where the sub-dentinal zone is wider (in my material it is at the sides of the tooth) the processes do not immediately enter the dentinal tubules; and if, as often happens, the processes are well separated from one another, the conditions for observing these very fine nerve filaments are ideal. Under very high magnifications these fine fibres, as they pass amongst the odontoblast processes appear, with their minute varicosities, like fine threads of spider web with adhering drops of moisture. The fine filaments give off collaterals which may undergo further branching. Eventually, unless they emerge from the plane of the microtome section, they terminate by definite end organs on the processes of the odontoblasts. The end organs are minute conical or leaf-like expansions of the terminal filaments; they

do not end "free" but are attached to the surface of the odontoblast process. As already remarked, these endings are to be seen mainly at the sides of the teeth, where conditions for precise observation are favourable. In my preparations they only occasionally appear in the crown region; but it would be incorrect to infer from this alone that they are comparatively uncommon here, for there are immense numbers of fine nerve fibres in the crown just under the odontoblast layer, for which no certain endings can be found.

Although terminal filaments may usually be seen ending in large numbers on the odontoblast processes in the sub-dentinal zone yet examples may sometimes be seen where a definite end-organ is situated at the entrance to a dentinal tube (e.g. fig. 4). Or again, a fibre may occasionally be found which actually appears to pass into the dentine (figs. 2, 5). On account of the deep staining of the lower margin of the dentine it is not possible to follow these particular fibres to their end-organs. But it does not seem probable that these occasional fibres pass far along the dentinal tubes, because immediately beyond the deeply staining margin they are no longer seen. It would be unjustifiable to look upon this as affording confirmation of Mummery's view of an innervation of dentine; the greater part of the dentine is completely free from visible nerve fibres and the few fibres that enter the tubes doubtless pass only a very short distance along them, probably ending there on the intratubular extension of the odontoblast process.

Variations from the above described condition are sometimes found. Occasionally comparatively coarse fibres may pass, without branching, past the layer of the odontoblast cells to the inner margin of the dentine. In the zone of the odontoblast processes they may turn and travel as quite coarse fibres along the under surface of the dentine, or they may branch into typical fine varicose filaments (fig. 6), giving off end-organs to the odontoblast processes. Sometimes, though not often, the nerves may form end-organs on the odontoblast processes without developing the fine filaments that run so characteristically for long distances below the dentine. An example is shown in fig. 3. Occasionally even comparatively coarse fibres are seen thus to terminate.

Although the endings of nerve fibres are most readily to be seen in the sub-dentinal zone of odontoblast processes, yet nerves appear to terminate also on the bodies of the odontoblast cells, where others have already described them. Many of the fibres that arise from the branching of axons below the layer of Weil are excessively fine varicose filaments, quite as delicate as those that are found in the sub-dentinal zone. They pass towards the odontoblast cells. But it is very difficult to follow them to their destination, for they do not stand out in sharp contrast against the background of strongly stained closely packed odontoblast cells. Nevertheless, in places where clearer observation can be made, apparently definite endings can be found for them. These appear merely as slightly enlarged varicosities or loops (fig. 7). They are not such definite end-organs as those already described on the odontoblast processes in the sub-

dentinal zone, and the possibility must be reckoned with that they are merely varicosities beyond which the staining of the fibres has suddenly failed. Thus in fig. 7 it is obviously very difficult to be certain that the globular endings of the fibres are genuine nerve end-organs. This is, of course, a difficulty that is commonly experienced in searching for the true endings of very delicate nerve filaments; for unless the endings are recognisable either by their relation to another cell, or by the presence of obvious end-organs there is no certain way by which we can discriminate between true endings and these apparent ones. Perhaps the most conclusive evidence that the nerve endings seen among the odontoblast cells are genuine endings and not the result of incomplete staining of fibres on their way to the sub-dentinal zone lies in the fact that sometimes nerves in this latter zone may form branches that actually grow back again into the pulp and evidently end there on the bodies of the odontoblast cells (fig. 2).

I have not been able to find instances of nerve terminals forming basket-works around the cells; nor have I seen any indication of nerve cells in the pulp, as described by Mummery.

The above observations then offer no support for the view that the dentine is itself traversed by nerve fibres. The evidence rests not merely on a failure to demonstrate nerves in the dentine, but on the fact that definite end-organs, associated with the odontoblasts can be demonstrated in the sub-dentinal zone. It would seem to follow therefore that the odontoblast processes in the dentine must be the receptor organs for these nerve endings; and if they can serve as receptors, then one can at present see no reason for inferring a special innervation of the dentine to account for clinical facts, when the microscopical evidence does not support it.

SUMMARY

1. The endings of nerves have been examined in Bielschowsky preparations of decalcified human molar teeth.

2. From the deeper region of the pulp nerves can be followed, right through the zone of odontoblast cells, into the sub-dentinal zone of odontoblast processes. Here they turn and can be followed as excessively fine, varicose branching filaments that run, often for considerable distances immediately under the dentine among the odontoblast processes. On these processes they terminate by definite end-organs which are attached to their surface.

3. Occasionally the nerves may end without forming these long varicose filaments.

4. Sometimes even quite coarse fibres may be seen running for long distances in the sub-dentinal zone.

5. Very delicate nerve filaments appear also to terminate by minute swellings (loops) on the bodies of the odontoblasts.

6. No evidence has been found for an extensive innervation of dentine, as described by Mummery and others. No nerve cells have been seen in the pulp.

Fig. 1.

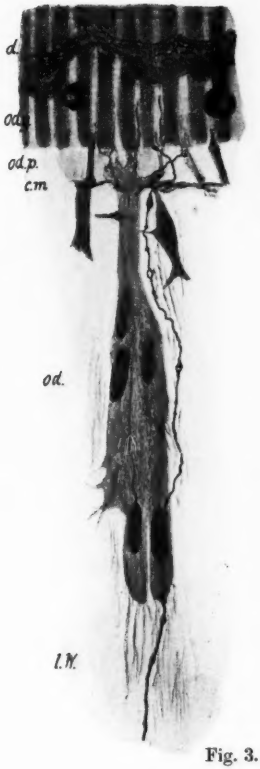

Fig. 3.

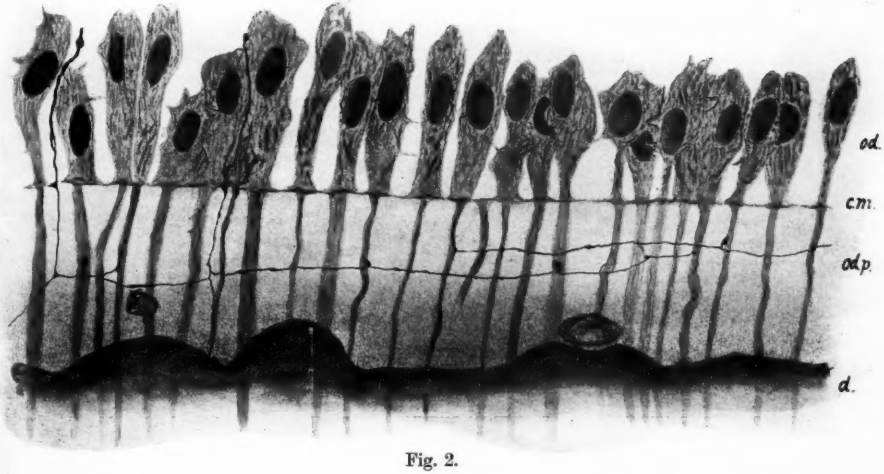

Fig. 2.

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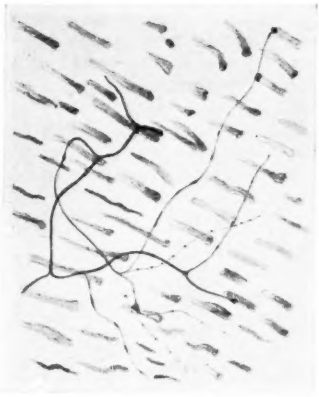

Fig. 6.

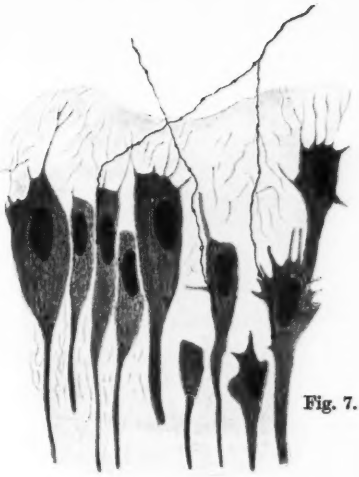

Fig. 7.

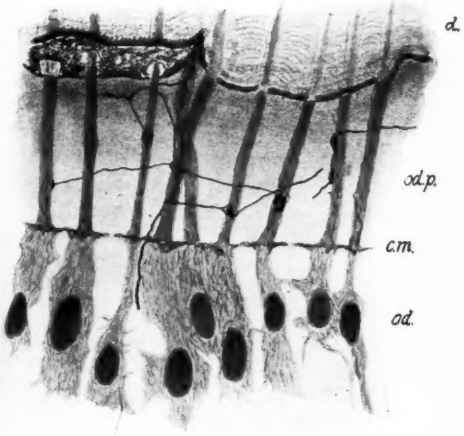

Fig. 4.

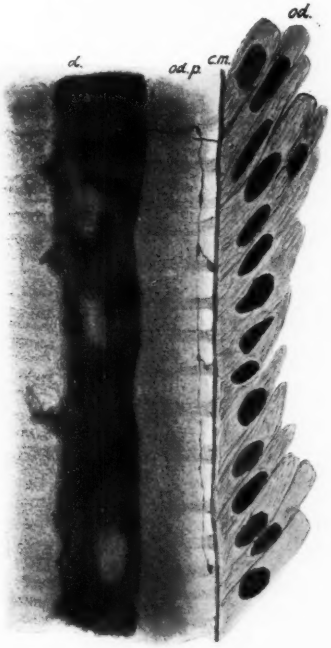

Fig. 5.

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EXPLANATION OF PLATES

The various zones at the junction of pulp and dentine are indicated thus: *d.* mature dentine; *odg.* odontogenic zone; *od.p.* sub-dentinal zone of odontoblast processes; *c.m.* "membrane" composed of the collars of the odontoblast cells; *od.* odontoblasts; *l.W.* layer of Weil.

Figs. 1–2 reduced by ½; figs. 4–7 reduced by ⅓.

PLATE I

Fig. 1. To show branching nerve fibres in the surface region of the pulp. The fine varicose fibres pass among the odontoblast cells; when examined under higher magnification it appears improbable that the "endings" of the fibres are the true terminations. Zeiss homog. immers. ⅛ in.; oc. × 15.

Fig. 2. A medium sized nerve fibre penetrates the odontoblast zone, branches in the sub-dentinal zone, and turning, runs as a delicate varicose filament among the odontoblast processes, on several of which it terminates by definite end-organs. A single short branch is seen to grow back among the odontoblasts, apparently to terminate there, whilst another branch passes to a dentinal tubule, but becomes obscured by the deeply staining lower margin of the dentine. Zeiss apochromat. 2 mm., oc. × 15.

Fig. 3. A comparatively coarse nerve fibre ending in the sub-dentinal zone without the formation of the usual type of long terminal filaments. Zeiss apochromat. 2 mm., oc. × 15.

PLATE II

Fig. 4. Fine nerve fibres branching among the odontoblast processes, and ending there by definite end-organs. Note the presence of an end-organ at the entrance to a dentinal tubule. Zeiss apochromat. 2 mm., oc. × 15.

Fig. 5. Fine nerve filaments with end-organs on the odontoblast processes in the sub-dentinal zone, towards the lower end of the tooth. A branch actually appears to enter a dentinal tubule but cannot be followed on account of the deep staining of the dentinal margin. Zeiss apochromat. 2 mm., oc. × 15.

Fig. 6. A comparatively coarse nerve fibre branching into fine nerve filaments among the odontoblast processes. Four end-organs are visible. Zeiss apochromat. 2 mm., oc. × 15.

Fig. 7. Fine nerve filaments apparently terminating on the bodies of the odontoblast cells. Zeiss apochromat. 2 mm., oc. × 15.

THE HISTOLOGY OF THE SPLEEN AND SUPRARENALS OF *ECHIDNA*[1]

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I. THE SPLEEN

The spleen of the Monotreme, remarkable on account of its form, was first described by Meckel in his classical monograph on *Ornithorhynchus*. The *Echidna* spleen has very much the same shape as that of *Ornithorhynchus*. In both species it differs very materially from the typical mammalian form, being tri-radiate or Y-shaped (text-fig. 1) and possessing a large pear-shaped swelling at the end of one of its limbs. The surface is indistinctly divided into irregular lobules by slight constrictions, whilst the hilum extends along its entire dorsal surface.

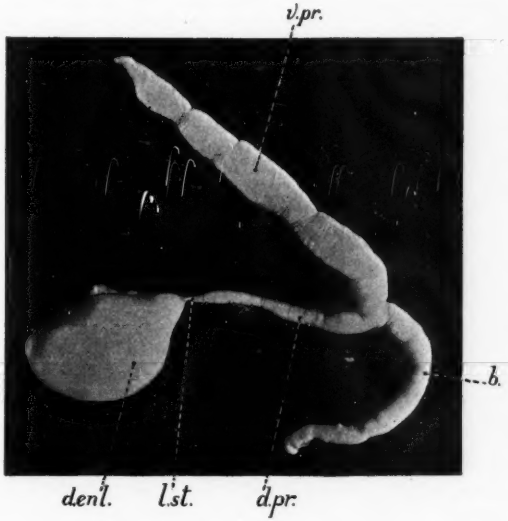
The spleen consists of three distinct parts, as can be seen in text-fig. 1. Of these, the body (*b.*) lies in the great omentum, along the greater curvature of the stomach, being directed towards the oesophagus. The process (*d.pr.*) bearing the large pear-shaped body (*d.enl.*) at its end, also lies in the great omentum and is directed dorso-laterally, extending as far as the distal colon, whilst the other process (*v.pr.*), also embedded in the great omentum, is directed ventrally. It is longer, thicker and more freely movable than the other two limbs of the organ.

The dimensions of the specimen examined were as follows: The body (*b.*) measured 4·5 cm. long and 0·5 cm. in diameter. The dorsal process (*d.pr.*) was about 3·5 cm. in length (excluding the bulbous portion) with a diameter of 0·5 cm. The pear-shaped portion (*d.enl.*) itself measured 3 cm. long, 2 cm. in the transverse plane and 1·5 cm. antero-posteriorly. These two parts of the dorsal process were connected together by a slender bridge of lymphoid tissue (*l.st.*). The ventral process (*v.pr.*) measured 7 cm. in length and 1 × 0·5 cm. in diameter.

The spleen was fixed in corrosive sublimate and was kept in alcohol for a good many years. It nevertheless proved to be extremely well preserved, and showed many interesting histological details. Serial sections of pieces from different regions of the organ were cut and stained by the following methods: Ehrlich's haematoxylin and eosin, Heidenhain's iron-haematoxylin

[1] Part of thesis approved for the degree of Ph.D. in the University of London.

and Van Gieson, Dominici, Pasini, Mann's methyl-blue eosin, Mallory's connective-tissue stain and Weigert's resorcin fuchsin. Special methods of staining, such as silver impregnation methods and the Prussian blue reaction for iron, could not be used. In order to supplement my observations on the *Echidna* spleen, I have examined the spleens of hedgehog, mouse, rabbit, *Hapale*, baboon, alligator, *Python*, the lizard, *Trachysaurus* and the Gymnophionan, *Siphonops annulatus*. These were fixed in a variety of fixatives (Zenker, Bouin, bichromate-formol, alcohol and formol) and stained by the methods mentioned above. My observations on these spleens are incorporated in the text.



Text-fig. 1. Photograph of the spleen of *Echidna*. Mag. $\frac{7}{10}$ times natural size. Note the triradiate appearance of the organ and the slight constrictions on its surface, giving rise to indistinctly divided irregular lobules. It consists of a body (*b.*), a ventral process (*v.pr.*) and a dorso-lateral process (*d.pr.*) which is connected with a pear-shaped enlargement (*d.enl.*) by means of a delicate stalk of lymphoid tissue (*l.st.*).

The supporting framework

(i) *The capsule and trabeculae*

The capsule which completely enclosed the organ consists of dense fibrous tissue, some elastic fibres and numerous smooth muscle cells. Arising from the capsule are strong trabeculae (Plate II, fig. 3, *T.*), similar in structure to the capsule, which form an anastomosing network in the interior of the organ. These trabeculae carry with them the blood vessels which run in them for some distance and eventually enter the splenic pulp, covered in a connective

tissue sheath, derived from the trabeculae—the so-called "hilar sheath" of Whitting (13).

The amount of smooth muscle, connective tissue and elastic fibres in the capsule and trabeculae varies considerably in the different spleens examined, as does the development of the trabecular system. For example, the spleen of the alligator possesses an extremely thick fibrous capsule, which is poor in muscle, and gives off no trabeculae, whilst that of *Siphonops* possesses neither capsule nor trabeculae. It is interesting to note that in these two forms, the reticular network is much more strongly developed, so that the reticular cells would appear to have taken on the supporting function of the trabeculae.

(ii) *The reticulo-endothelial framework*

The supporting framework of the splenic pulp, which occupies the meshes of the capsule-trabecular system is formed of a uniform spongy framework, composed of branching reticular cells (Plate II, fig. 3, *ret.c.*) which are closely applied to the reticular fibres (*ret.f.*). This loose network is infiltrated with the blood cells (*r.bl.*) which give rise to the characteristic red colour of the splenic pulp. The delicate reticular fibres (*ret.f.*), as can be seen in Plate II, fig. 3, become directly continuous with the collagen fibres (*c.t.*) forming the capsule, trabeculae and tunica adventitia of the blood vessels, whilst some of the reticular fibres appear to be attached to the smooth muscle fibres (*s.m.*) present in these regions.

(iii) *The vascular system*

The arteries and the veins enter and leave the spleen at the hilum. This lies along the whole length of the organ on its dorsal surface. The arteries at first run in the trabeculae, but they soon pass into the splenic pulp itself, enclosed in a connective tissue sheath—the "hilar sheath" derived from the fibrous trabeculae. In the pulp, the "hilar sheath" becomes infiltrated with small lymphocytes, forming a lymphoid coat round the vessel, the white pulp or Malpighian corpuscle. The Malpighian corpuscles vary in size, but they are frequently large, running for more than 5 mm. along the arteriole, whilst they are not of uniform thickness throughout. Several such corpuscles may occur at varying intervals along the length of an arteriole.

As is well known, the form of this lymphoid sheath varies in different spleens. In the alligator and *Trachysaurus*, the arterioles are enclosed in a uniform lymphoid sheath throughout their entire extent, whilst in *Siphonops* such lymphoid sheaths are also found along the veins. In *Python*, on the contrary, the vessels are completely devoid of a lymphoid covering.

In *Echidna*, the Malpighian corpuscles (*M.c.*), as can be seen in Plate I, fig. 1, are surrounded by a thin capsule of condensed reticular fibres, in which also occur smooth muscle fibres, and collagen fibres, derived from the "hilar sheath." The artery (text-fig. 2, *c.a.*), after running a short distance through the corpuscle, may divide dichotomously, and some of the branches (*a.*) so

formed, may immediately leave the corpuscle and run into the red pulp, where they develop fresh corpuscles (*M.c.*), whilst other branches, the smaller, run right through the corpuscles and open into the reticulum of the red pulp. The majority of these branches, however, do not reach the reticulum, for their endothelial walls break down and the blood is poured into the lymphoid tissue, forming a blood sinus (Plate I, fig. 1, *bl.s.*) round the periphery of the corpuscle and enclosed by the reticular capsule (Plate I, fig. 1, *cap.M.*). The blood sinus (Plate I, fig. 1, *bl.s.*) is not an endothelially lined space, but the blood actually lies free in the reticulum, from which it is eventually drained into the surrounding pulp (*sp.p.*) by means of endothelially lined capillaries (*c.*). This peripheral blood sinus is absent in the spleen of the young animal, and it may be that the sinus is concerned with the increased blood destruction in the older animal. In the latter there is present chiefly in the sinuses, but also in the pulp, a considerable number of red blood cells undergoing haemolysis which were not found in such quantity in the younger animal.

The main arteriole eventually leaves the Malpighian corpuscle and runs into the red pulp, where it divides into two or more penicillate arterioles (text-fig. 2, *p.a.*) which acquire dense, though ill-defined sheaths or ellipsoids (*el.*) around them.

In *Echidna*, the ellipsoids (Plate II, fig. 4, *el.*) consist mainly of longitudinally arranged condensed reticular fibres (*ret.f.*) intermixed with large reticular cells (*ret.c.*). The penicillate artery (*p.a.*) is lined by an irregular layer of endothelial cells (*end.c.*) which possess scanty protoplasm and large nuclei bulging into the lumen (*l.*). Peripherally the dense reticulum of the ellipsoid gradually merges into the reticulum of the pulp. The penicillate artery does not branch in the ellipsoid and no canaliculi, such as are described by Bannwarth (1) and more recently by McNee (8) can be seen. In the matrix of the ellipsoid, however, there are visible isolated red blood corpuscles which suggest that such canaliculi may exist.

The ellipsoids are better developed in the spleen of the alligator and their structure can be more easily studied. The penicillate artery here divides into four or five smaller branches within the fibrous sheath, producing a quadri- or penta-cornuate ellipsoid, whilst outside this, enclosing the penicillate artery and its ellipsoids, is a lymphoid sheath. This lymphoid covering to the ellipsoid appears to be peculiar to the spleen of the alligator, and I have found nothing comparable to it in any of the spleens I have examined.

In the spleens of the baboon, *Hapale*, rabbit and mouse, which have well-developed Malpighian corpuscles, the ellipsoids are poorly developed, so that I can confirm the statement of McNee (8) that as a general rule, in animals with well-developed Malpighian corpuscles, the ellipsoids are poorly developed. Further, in this connection, Bannwarth (1) observed that during development the ellipsoids appear before the Malpighian corpuscles and that they become indistinct later in embryonic life, when the Malpighian corpuscles make their definite appearance.

The mode of termination of the penicillate arteries is still a matter of controversy. Helly (3) and Thoma (11) believe that there is a closed circulation in the human spleen and that the penicillate arteries open directly into the venous sinuses, but that some of the blood passes into the pulp through openings in dilated terminations of the arteries—the so-called "ampulla." These openings, according to Thoma (11), are very minute, whilst Helly (3), on the other hand, believes that they are of considerable size. Many other workers, Kultschitsky (4), Mall (6) and Sobotta (10), believe that the circulation is an open one, whilst Wiedenreich (12) states that both forms of circulation exist in the same spleen.

Mollier (7) more recently has made a further study of the structure of the venous capillaries and sinuses in sheep and Man, and he showed that they are provided with innumerable stomata, through which a considerable amount of blood can pass from the pulp during the passage of blood from the arterial to the venous side. Neubert (9) has further shown that the same holds good also in dog, cat and pig.

In *Echidna*, the penicillate artery (Plate II, fig. 4, *p.a.*) after leaving the ellipsoid becomes funnel-like, its endothelial lining (*end.c.*) flattens out and gradually becomes indistinguishable from the surrounding reticular cells (*ret.c.*). The blood thus passes directly into the red pulp, so that the circulation appears to be of the open variety. In addition, however, some arterioles (text-fig. 2, *p.a.*1) run directly through the pulp (*sp.p.*) to open into the venous sinuses (*v.s.*), but this is not of frequent occurrence.

Certain spleens, those of the alligator, *Siphonops*, hedgehog and *Hapale*, which I have examined, possess an open circulation, whilst in others, those of *Python* and *Trachysaurus*, the circulation is closed, as McNee (8) has stated.

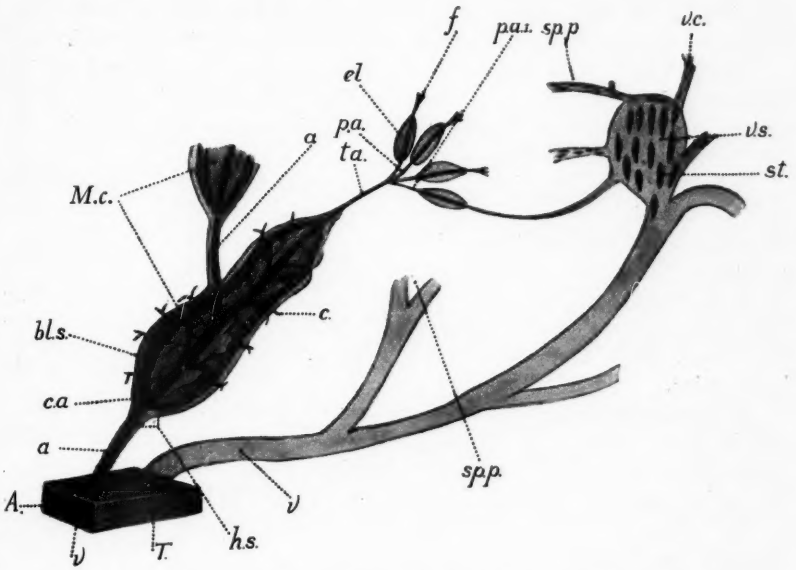
The blood is eventually collected from the pulp by small capillaries, which open into the venous sinuses (Plate II, fig. 5, *v.s.*). The origin of these venous capillaries has been much discussed. Thoma (11) described the venous capillaries as being continuous with the distal part of the ampulla, whilst Kultschitsky (4) thought that they arise directly from the pulp spaces. Mollier (7), on the other hand, describes the blood cells passing to the venous sinuses, through stomata in the walls of the sinuses.

In *Echidna* the venous capillaries originate in the reticulum of the red pulp and possess an incomplete endothelial lining, like that of the venous sinuses, so that blood drains into them from the surrounding tissue. These capillaries, after a longer or shorter course in the pulp, run into the venous sinuses.

The general scheme of the circulation of blood in the *Echidna* spleen is given in the accompanying text-fig. 2.

(iv) *Cytology of the spleen*

The cellular elements forming the red pulp, apart from those of the supporting framework, comprise the cells of the blood, macrophages and lymphoid elements. In some animals, e.g. mouse and hedgehog, the red pulp contains myelocytes, megakaryocytes and plasma cells in addition.



Text-fig. 2. As can be seen, the artery (*A.*), on leaving the trabecula (*T.*) enters a Malpighian corpuscle (*M.c.*), wherein it gives off numerous branches. Some of these enter the peripheral blood space (*bl.s.*) of the corpuscle (*M.c.*), the blood from which eventually drains into pulp (*sp.p.*), whilst others leave the corpuscle to form penicillate arteries (*p.a.*) and these either open directly into the pulp by funnel-like openings (*f.*) or pass (*p.a.*1) to open directly into the venous sinuses (*v.s.*). From the pulp (*sp.p.*) the blood passes into the venous capillaries (*v.c.*) or through the stomata (*st.*) in the walls of the venous sinuses into the latter, and thence into the veins (*v.*).

(*a*) *Cells of the supporting framework, including the histiocytes (the so-called reticulo-endothelial system).*

The connective tissue cells, plain muscle cells and plasma cells, which are found in the capsule and the trabeculae, are so well known, that it is not necessary for me to do more than mention them here. The reticular cells (histiocytes) on the other hand, present a more complex problem.

These cells, as I have already mentioned, form the reticular framework of the splenic pulp, in which reticular fibres can be seen when stained by appropriate methods, such as Mallory's or Pasini's stains. By means of Foot

and Mènard's silver impregnation method (2), still clearer pictures of this reticulum can be obtained, but unfortunately this method could not be used on the *Echidna* spleen.

The reticular cells are large and are provided with irregular protoplasmic processes, which unite with similar processes of the neighbouring cells to form a syncytial network, in which the reticular fibres are embedded. These cells possess a granular acidophil cytoplasm and contain one or two oval or spherical nuclei, with well-defined nuclear membranes, rather scanty chromatin, clumped into one or two large granules and one or two nucleoli.

Such reticular cells also line the venous sinuses. They differ from the ordinary endothelial cells by the fact that they project into the lumen of the sinuses and are actively phagocytic, whilst their processes are often in continuity with the syncytial network.

In the spleens of the alligator, *Siphonops*, hedgehog and *Echidna* (although to a less extent in the *Echidna* spleen than among the others I have mentioned), macrophages are formed from the reticular cells. These macrophages separate off from the reticulum and become actively phagocytic, ingesting pigment or other cell débris. They measure up to 10μ in greatest diameter and possess oval nuclei with rather scanty chromatin and a prominent nuclear membrane, whilst their cytoplasm is abundant and is stained faintly with basic stains.

Pigment may also be seen in the reticular cells lining the venous sinuses, and is very noticeable in those of *Siphonops*, whilst extracellular reddish brown pigment, exhibiting a well-marked iron reaction with Prussian blue, was noticed round the ellipsoids in the spleens of the alligator and the hedgehog. In the spleen of *Echidna*, however, neither extracellular nor intracellular pigment was observed.

(*b*) *Cells of the lymphoid series.*

In *Echidna*, the Malpighian corpuscles are formed entirely of small lymphocytes and no germ centres are present.

(*c*) *Blood constituents.*

Blood cells are found scattered in large numbers throughout the red pulp as well as in the blood sinus round the periphery of the white pulp. Red blood cells were observed in process of fragmentation and were in course of ingestion by macrophages, whilst others had undergone haemolysis and their débris was seen lying in the venous sinuses. No erythropoiesis was taking place in the spleen.

(*d*) *Special cells.*

In the pear-shaped enlargement of the dorsal limb, certain large cells are found scattered in the reticular network of the red pulp (Plate II, fig. 6, *gr.c.*). They measure up to 0·016 × 0·012 mm. in diameter, and are remarkable in that their cytoplasm is filled with coarse acidophil granules (*gr.*), spherical

in shape and in such abundance that the nucleus is displaced to one side of the cell and presents a spherical pycnotic appearance (*p.nuc.*). Similar cells are also found in the spleen of the alligator, *Siphonops* and mouse, although in these animals the cells are smaller, whilst the granules are less abundant and the nucleus is round and vesicular. Such cells were also seen in the spleens of the rabbit, *Hapale* and baboon, but they are extremely rare. I can find no reference to such cells in the literature.

Megakaryocytes, such as occur in spleens of the hedgehog, rabbit and mouse were not found in *Echidna*.

Summary

1. The spleen of the *Echidna* is tri-radiate in form, with a pear-shaped enlargement at the end of one of its limbs.

2. It is invested by a well-developed fibrous capsule, which contains a considerable quantity of smooth muscle and which sends trabeculae into the splenic pulp.

3. The stroma of the gland consists of a branching syncytium formed by the reticular cells, in which run reticular fibres. These reticular fibres are continuous with the collagen fibres of the capsule and trabeculae, and are also attached to the smooth muscle cells.

4. The Malpighian corpuscles which are developed round the arterioles are characterised by the presence of a large blood sinus round their periphery.

5. The penicillate arterioles possess ellipsoids, each of which consists of a condensation of reticular cells and fibres. On leaving the ellipsoid, the penicillate arteriole becomes funnel-shaped and opens into the splenic pulp or into a venous sinus.

6. Venous capillaries and sinuses are lined by an incomplete layer of reticular cells, so that there are numerous openings in their walls, through which the red blood cells can pass directly into their lumina.

7. The cellular elements of the pulp comprise reticular cells, macrophages derived from the reticular cells and blood cells. In the bulb-like enlargement of the dorsal limb there were also present large coarsely granular acidophil cells, the origin of which is unknown. Giant cells were not observed.

II. THE SUPRARENAL

The Monotreme suprarenal has been described by Elliott and Tuckett (14). According to their account in both *Ornithorhynchus* and *Echidna*, it consists of a cortex and medulla, distinct from each other and separated by a layer of connective tissue and a sinus-like "blood space." The cortex forms the major portion of the gland and surrounds the medullary tissue except at the caudal end; in *Ornithorhynchus* it is divided into lobules by the blood vessels passing into it from the capsule. The cells on the edge of these lobules are small and

closely packed together, but those in the centre of the lobules are larger and coarsely vacuolated. This lobulation is not found in the narrow zone immediately adjoining the medulla. In *Echidna* the cortex is not lobulated and the cells are arranged in anastomosing columns, in the meshes of which are found large sinus-like capillaries. At the caudal end of the gland prolongations of the cortical tissue are found intermixed with the medullary cells, whilst small ganglionic masses are found lying near the medullary tissue or actually embedded in it. The material used by these workers was not well preserved, so that they do not give any detailed account of the structure of the cortical or medullary cells.

Material

The suprarenals were fixed in formalin and kept in alcohol for a number of years. After embedding in paraffin, serial sections were cut along the long axis of the gland and sections were stained with Ehrlich's haematoxylin and counterstained with eosin, Pasini, Dominici, with Masson's triple stain and Dustin's silver impregnation method. In order to supplement my observations I have examined the suprarenals of pigeon, rabbit, guinea-pig, dog, cat, ox and Man, which were lent to me by Miss R. Deanesly.

Observations

The suprarenal in *Echidna* lies on the ventro-lateral aspect of the corresponding kidney, enclosed with this organ in a fold of the visceral peritoneum. It is elongated or oval in form, measuring in cross-diameter approximately 1·2 × 0·65 cm. and is invested with a thick fibrous capsule.

As can be seen in text-fig. 3, the medulla (*chr.c.*) forms an elongated mass, surrounded by the cortical cells (*z.r.*2, *z.g.*) except over the caudal pole of the gland where the medulla comes to the surface (*h*). The blood vessels enter and leave the gland in this region and a small ganglion (*g.n.*) is also often found embedded in the medullary tissue (*chr.c.*). The cortex and medulla are more or less distinctly separated from each other by a layer of connective tissue and blood sinuses (text-fig. 4, *c.t.*, *bl.s.*). In the caudal region prolongations of the cortical tissue are found among the chromaffin cells.

The cortex

The cortex, as can be seen in text-fig. 3, forms the anterior or cranial part of the gland, comprising about two-thirds of the entire organ. It envelops the medulla (*chr.c.*) except at the caudal pole of the gland, and it differs somewhat in its histological structure in the cranial and caudal portions. In the cranial region, the cortex consists of three zones (text-fig. 3, *z.r.*1, *z.f.*, *z.r.*2), the outer zone (*z.r.*1) and the deepest zone (*z.r.*2) appearing reticular in form and resembling the zona reticularis of the Eutherian organ. These two zones become continuous with each other in the caudal part of the gland (Plate III,

fig. 7, *j.*), whilst between these two layers is found a more compact inner zone (text-fig. 3, *z.f.*), the zona fasciculata of the Eutheria.

The appearance presented by the cortical tissue here is suggestive of an invagination caused by the approximation of the medullary tissue to the cortical cells. The deepest layer of the cortex lying next to the medulla being originally part of the outer cortical layer, whilst the inner compact layer formed the centre of the originally spherical cortical mass (text-fig. 3, *chr.c.*).

The medulla is covered by a prolongation of the zona reticularis of the cortex (text-fig. 3, *z.r.*1) which spreads over the chromaffin tissue except at the caudal pole. Round the hilum there is present an area of cortical tissue which differs in histological detail from that forming the other cortical zones. This layer resembles the zona glomerulosa of the Eutherian suprarenal, and I propose to use this name for it. The cells of this layer (text-fig. 3, *z.g.*, Plate III, fig. 8, *z.g.*) spread forwards cranially as a thin sheet between the capsule and the outer reticular zone, and gradually thin out so that they are absent from the central and cranial parts of the gland. Sometimes, however, isolated groups of cells belonging to this zone can be recognised under the capsule in these regions. In the region of the hilum this layer becomes infiltrated with connective tissue and round the hilum itself (text-fig. 5, *h.*) the connective tissue almost completely replaces the cortical cells.

The outer reticular zone of the cortex (text-fig. 3, *z.r.*1) lies directly under the thick fibrous capsule and forms about two-thirds of the cortical tissue of the gland. It consists of an anastomosing network (Plate III, fig. 7, *z.r.*1) formed by columns of polygonal-shaped cells which are indistinctly delimited from each other. Between the cell columns are spaces which are occupied by blood vessels (Plate III, fig. 9, *c.*) and connective tissue, derived from the septa which grow in from the capsule. The cells forming the cell columns have vacuolated cytoplasm, whilst their nuclei are small and spherical, and stain intensely with haematoxylin. This zone resembles the zona reticularis of the suprarenal of the Eutherian Mammal in its histological detail, although it differs from this in its position directly under the capsule.

At the cranial end of the gland is found a peculiar conical area (text-fig. 3, *c.pt.*) which projects into the connective tissue of the capsule (*cap.*). In this region the connective tissue of the capsule appears lamellar in structure, and in the meshes of this are found groups of cells which stain somewhat darker than those of the outer layer of the cortex, although in general detail they resemble these cells. They form small groups or lamellae of irregular size which are separated from each other by well-defined connective tissue septa. Blood vessels characteristic of the outer cortical zone are not present.

The inner zone of cells or zona fasciculata (Plate III, fig. 10, *z.f.*) consists of rather coarse strands of paler staining polyhedral cells which run more or less parallel to each other and which are separated by reticular fibres (Plate III, fig. 10, *ret.f.*) and small capillaries. These cells are larger than those of the outer layer and stain less intensely. Their nuclei (*nuc.*) are round, whilst the

chromatin is clumped in small granules lying close to the nuclear membrane. The cytoplasm contains vacuoles, larger than those of the outer layer, so that it seems likely that this region is rich in lipoids.

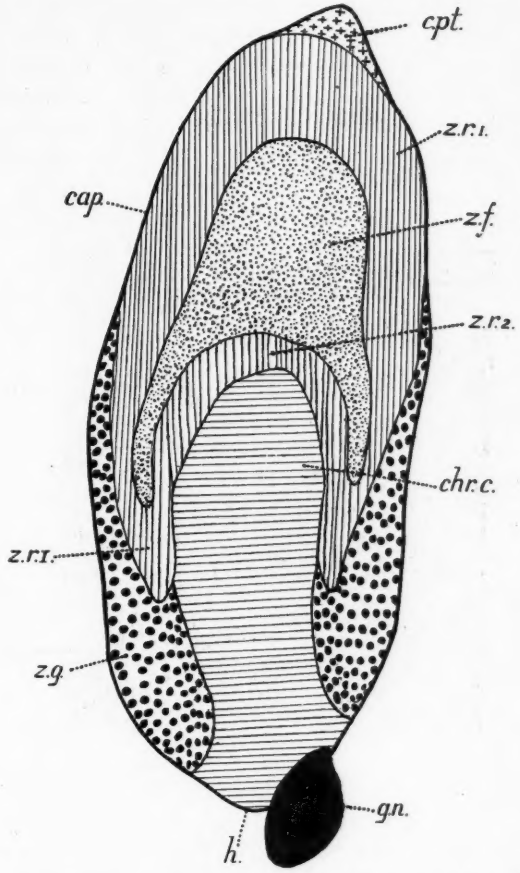
Separating this zone from the medullary tissue is a region composed of anastomosing strands of cells (Plate III, fig. 7, *z.r.*2) which resembles the zona reticularis (*z.r.*1) in its histological structure. This zone is considerably thinner than the outer reticular zone (*z.r.*1), although it becomes continuous with that in the caudal part of the gland. These two layers of the zona reticularis form a covering to the medullary tissue in the caudal part of the gland. Towards the hilum, however, they are replaced by a layer of darkly staining, coarsely vacuolated cells forming the zona glomerulosa (text-fig. 3, *z.g.*) which for a short distance spreads anteriorly over the cells of the zona reticularis and lies between this zone and the connective tissue capsule. These cells of the zona glomerulosa are arranged in irregular or oval groups (Plate III, fig. 11, *z.g.*) which are separated from each other by anastomosing connective tissue septa (*c.t.*) derived from the capsule (*cap.*). These connective tissue septa become very numerous in the region of the hilum and often completely replace the cortical cells. The cells are columnar in shape (Plate III, fig. 11, *z.g.*) and distinctly separated from each other, whilst their nuclei are oval or spherical, and stain deeply with haematoxylin. This zone, as I have already described, spreads over the cortical cells towards the middle of the gland, whilst isolated groups of these cells can be often seen in the more cranial part of the gland.

The medulla

The medulla consists of cords of cells (text-fig. 4, *chr.c.*) separated by fine strands of reticular connective tissue (*ret.f.*). The latter anastomose to form a network, in the meshes of which are found blood capillaries and blood sinuses.

The chromaffin tissue consists of large polyhedral cells which are nearly twice as large as the cortical cells, whilst they are not sharply delimited from each other. Their nuclei (*nuc.*) are large, spherical in shape, with a prominent nuclear membrane, whilst the chromatin is aggregated into one or two granules. The cytoplasm is finely granular and contains numerous large vacuoles. These, as far as I am aware, have not been described in any other animal.

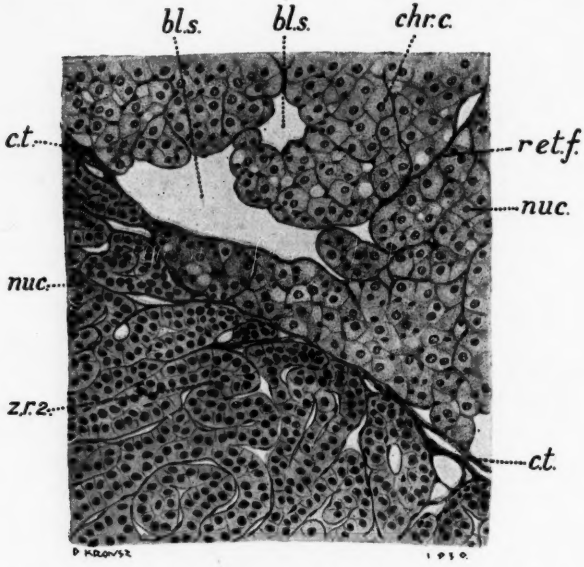
The medulla comes to the surface at the hilum of the gland (text-fig. 5, *h.*) and in this region just as Elliott and Tuckett (14) described there is present a small nerve ganglion (*g.n.*). This ganglion is enclosed in a connective tissue capsule which separates it from the medullary cells. The nerve cells (*n.c.*) are multipolar and their processes ramify amongst the cells of the chromaffin tissue.



Text-fig. 3. Diagram to show the relation of the cortex and medulla in the suprarenal gland of *Echidna*. The cortex can be seen to form the cranial part of the gland and it forms a covering to the medulla (*chr.c.*) except over the caudal pole of the gland. The cortex consists of three zones (*z.r.*1, *z.f.*, *z.g.*). Of these the zona reticularis (*z.r.*1) and the zona fasciculata (*z.f.*) are found aggregated at the cranial end of the gland. The zona reticularis also forms a narrow zone (*z.r.*2) adjacent to the medulla which is continuous with the outer zone (*z.r.*1) in the caudal part of the gland. The zona glomerulosa (*z.g.*) forms a thickened mass at the caudal end of the gland which runs forwards for a short distance over the rest of the cortex towards the middle of the gland. The medulla (*chr.c.*) is oval in shape and projects on the surface at the caudal pole where a small sympathetic ganglion (*g.n.*) is found embedded in the medullary tissue. On the cranial end is found a small conical area (*c.pt.*) formed by cells arranged in groups or lamella between the layers of the capsule.

The supporting framework

The suprarenal is invested by a thick fibrous capsule which is formed of two parts, an outer compact layer of fibrous connective tissue and an inner looser layer composed of reticular cells and fibres. The capsule becomes continuous with the connective tissue covering the blood vessels in the region of the hilum, and in the cranial region it forms a covering to the small conical projection which I have shown in text-fig. 3, *c.pt.* The septa which run into

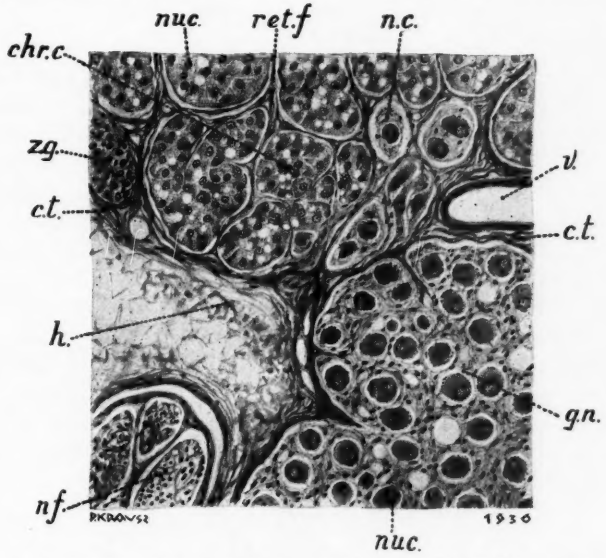


Text-fig. 4. High-power drawing of the suprarenal of *Echnida*, showing the medulla (*chr.c.*) and the zona reticularis (*z.r.*2) lying adjacent to it. Mag. × 130. The chromaffin cells (*chr.c.*) are arranged in irregular cords, separated by strands of reticular fibres (*ret.f.*). The nuclei (*nuc.*) of these cells are large and spherical in shape, whilst the cytoplasm contains large vacuoles. Blood sinuses (*bl.s.*) are also seen in the chromaffin tissue. The zona reticularis (*z.r.*2), formed of anastomosing cell-cords, lies separated from the medulla (*chr.c.*) by a layer of connective tissue (*c.t.*). These cells are smaller than those of the medulla and stain darker with haematoxylin.

the cortex from the capsule are of two types, the larger ones extend through the greater part of the cortex and are formed of connective tissue and reticular fibres, whilst the smaller ones are formed chiefly of reticular fibres. These latter spread round the cell columns in the zona reticularis, whilst the longer septa reach the zona fasciculata, where they separate into thin strands or threads which ramify round the cellular strands. The reticular fibres are not so coarse here as in the zona reticularis, whilst the cellular strands are more closely packed together.

In the zona glomerulosa round the caudal part of the gland, the connective tissue septa are much coarser than in the other cortical zones, and they ramify between the cortical cells, separating them into small, round or oval masses (Plate III, fig. 11, *z.g.*). Towards the hilum, the invasion of the cortical zone by the connective tissue is so great that the cortical cells form isolated groups among the connective tissue strands and they are often almost completely replaced by dense fibrous connective tissue.

In the deepest cortical zone (i.e. the zone continuous with the outer reticular zone which lies adjacent to the medullary tissue), the septa are thicker



Text-fig. 5. High-power drawing of caudal end of the suprarenal. *Echidna*. Mag. × 115. The medulla (*chr.c.*) comes to the surface of the gland in this region (*h.*) and close to it can be seen a small sympathetic ganglion (*g.n.*). Some of the nerve-cells (*n.c.*) of this ganglion can be seen embedded in the chromaffin tissue (*chr.c.*). A bundle of nerve fibres (*n.f.*) can be seen in the left lower corner of the figure.

and spread out between the cells. They run into the connective tissue layer between the cortex and medulla.

The supporting framework of the medulla is derived partly from the layer of connective tissue (Plate III, fig. 7, *c.t.*) which separates the cortex and the medulla, partly from the connective tissue of the hilum and partly from the connective tissue surrounding the prolongations of cortical tissue which run into the medulla. The connective tissue and reticular fibres (text-fig. 5, *ret.f.*) spread out between the cell strands, forming a well-defined network round them. This network is much coarser and better developed than in the cortical regions.

The blood supply (text-fig 6)

The suprarenal receives its blood from an artery (*a.*) which runs into the organ at the hilum. This vessel divides into numerous branches, some of which (*a.cap.*) ramify in the capsule, whilst the majority run directly to the cortex (*a.cor.*) and the medulla (*a.chr.c.*).

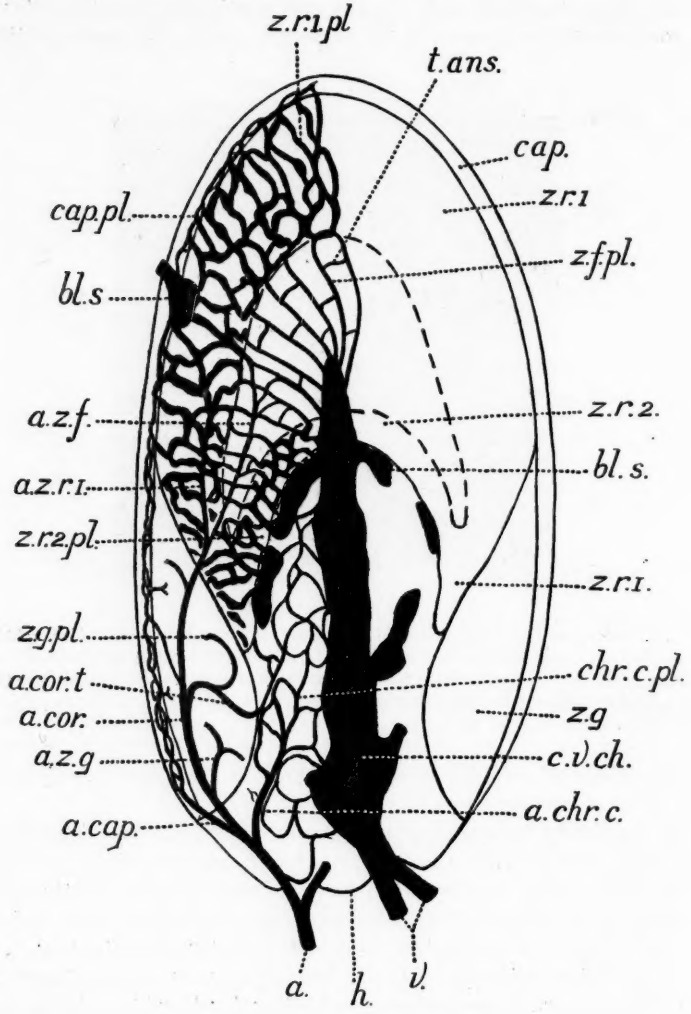


Fig. 6 (see legend opposite).

(1) *Arteries of the capsule* (*a.cap.*)

The capsule is supplied by a number of small branches from the main artery (*a.*) which form an ill-defined capillary plexus (*cap.pl.*) in the inner connective tissue layer of the capsule. This capillary plexus is very irregular and is best developed in the conical projection at the cranial end of the gland. The blood from the capillaries is collected into large sinuses (*bl.s.*) from which it runs into the surrounding connective tissue. Some of the blood from the capillary plexus also drains from the cortex into the cortico-medullary blood sinuses (*bl.s.*) and the central venous channel (*c.v.ch.*).

(2) *Arterioles of the cortex* (*a.cor.*)

The cortex is supplied by numerous small arterioles (*a.z.r.*1, *a.z.f.*, *a.z.g.*) which run into the cortical substance direct from the hilum. In the cortex the capillaries derived from these arterioles run into the connective tissue septa derived from the capsule, and ramify between the anastomosing cellular strands forming a complicated sinus-like plexus (*z.r.*1.*pl.*).

In the outer zone the capillaries form wide anastomosing channels, the size of the vessels being dependent on the mesh-like character of the spaces between the cellular strands. In the inner zone where the cell strands are thicker and more uniformly arranged, the capillaries (*z.f.pl.*) run parallel to one another, whilst they are joined by numerous transverse anastomoses (*t.ans.*). On reaching the deeper zone adjacent to the medullary tissue the capillaries (*z.r.*2.*pl.*) become larger and form an irregular meshed plexus.

Some of the larger arterioles (*a.z.f.*) which run through the outer reticular zone to the zona fasciculata are accompanied by an invagination of cells of the zona reticularis which form a thin covering to the vessels. This covering has been observed by Elliott and Tuckett (14) in the suprarenal of *Ornithorhynchus*.

Text-fig. 6. Diagram to show the blood-supply of the suprarenal. *Echidna*. Note the suprarenal is supplied by an artery (*a.*) which enters the gland at the hilum and divides into numerous branches (*a.cap.*, *a.cor.*, *a.chr.c.*). Some of the branches (*a.cap.*) enter the capsule (*cap.*) and form a capillary plexus (*cap.pl.*) in the deepest layer. The blood from these capillaries may drain into the subcapsular blood-sinuses (*bl.s.*) and so into the surrounding connective tissue and away from the gland or into the capillaries (*z.g.pl.*, *z.r.*1.*pl.*, *z.f.pl.*, *z.r.*2.*pl.*) of the cortex. Other branches (*a.cor.*) pass directly into the cortex and run in a longitudinal direction through this giving off numerous small branches (*a.z.g.*, *a.z.r.*1, *a.z.f.*) which ramify in the connective tissue septa between the cells and form a capillary plexus (*z.r.*1.*pl.*, *z.r.*2.*pl.*, *z.f.pl.*, *z.g.pl.*). In the zona reticularis (*z.r.*1, *z.r.*2), the capillaries (*z.r.*1.*pl.*, *z.r.*2.*pl.*) are large and irregular in shape, whilst in the zona fasciculata, they (*z.f.pl.*) are narrow and lie parallel to one another, joined by numerous transverse anastomoses (*t.ans.*). The blood from the superficial cortex may drain into the subcapsular blood-sinuses (*bl.s.*) of the cortico-medullary zone or into the central venous channel (*c.v.ch.*).

The medulla is supplied by blood from two sources, by branches (*a.chr.c.*) from the main artery (*a.*) or indirectly from capillaries (*a.cor.t.*) derived from the cortical branches (*a.cor.*) of the main vessel (*a.*) which runs along cortical trabeculae. On reaching the medulla they form a capillary plexus (*chr.c.pl.*) which is ultimately drained into the blood sinuses (*bl.s.*) of the cortico-medullary zone or into the central venous channel (*c.v.ch.*). At hilum (*h.*) the central venous channel (*c.v.ch.*) gives rise to two or more veins (*v.*).

The blood from the capillaries of the zona reticularis (*z.r.l.pl.*) drains either into subcapsular sinuses or through the capillary plexus of the inner zones into the central venous channel (*c.v.ch.*) which runs from the centre of the cortex through the medulla to the hilum. In the deeper zones the blood drains either into the small irregular blood sinuses (*bl.s.*) of the cortico-medullary boundary zone or into the central venous channel (*c.v.ch.*).

(3) *Arterioles of the medulla* (*a.chr.c.*)

The medulla is supplied by small arterioles (*a.chr.c.*) entering it directly from the hilum or by capillaries (*a.cor.t.*) which run into it along the cortical trabeculae. On reaching the medulla these vessels form an irregular plexus (*chr.c.pl.*) in the connective tissue reticulum round the cell columns. The vessels of this plexus are very much larger than those in the cortex and they drain either into the central venous channel (*c.v.ch.*) or into the large sinus-like branches (*bl.s.*) of this channel. From this channel the blood is collected into veins (*v.*) in the region of the hilum. The venous blood therefore from the cortex and medulla are intermixed in this central venous channel (*c.v.ch.*), for although a number of venules run into the capsule from the superficial zone of the cortex, most of the blood is collected in this central venous channel (*c.v.ch.*).

The walls of the capillaries, blood sinuses (*bl.s.*) and the central venous channel (*c.v.ch*) are formed by a single layer of endothelium resting on the reticular fibres. In the region of the hilum (*h.*) the veins (*v.*) which arise from the blood sinuses are enclosed in a single layer of longitudinally running smooth muscle fibres. A circular muscle coat was always absent from these vessels. This peculiar arrangement of the muscle coat on these veins has also been described by Flint (15) in the suprarenal of the dog.

Discussion

The suprarenal of the Monotreme forms an intermediate stage between that found in the lower Vertebrates and that of the higher Mammalia. In the Bird and in some Reptiles the cortical and medullary cells are found intimately intermixed with each other. This is perhaps best seen in the Bird's suprarenal, where the entire organ is formed by an intermixing of cortical and medullary tissue; in the Reptilia the intermingling of the two layers is less than in the Bird; and in the lizard and snake, according to Poll (16), there is a separation of the cortical tissue to the dorsal surface of the gland. In the cortical region, the medullary tissue is still intermixed with strands of cortical cells.

In the Monotreme suprarenal, this separation is almost complete, for the medullary tissue is aggregated at the caudal end of the gland, whilst the cortical cells form a covering to the medullary cells except over the caudal

pole. In this region, cellular trabeculae run into the medulla from the cortex, but the intermingling of the cortical and medullary tissue is of very slight extent compared with that in the Reptilia.

The appearance presented by the *Echidna* suprarenal is suggestive of the invagination of the cortical tissue by the medullary cells, for, as can be seen in text-fig. 3, the outer zona reticularis is continuous with the inner reticular zone of the cortex which lies adjacent to the cortico-medullary boundary. At the caudal end of the gland the zona reticularis has secondarily, so it appears, grown round the medullary tissue to enclose it except over the caudal pole. The zona glomerulosa appears only at the caudal end of the gland and grows forward cranially for a short distance over the zona reticularis.

It is interesting to note that in the bird (pigeon), the cortical cells resemble in appearance the cells of the zona glomerulosa of the Eutherian Mammals. Cells comparable to those of the zona fasciculata and zona reticularis are not found in the lower forms. These zones can be distinguished in the Monotreme suprarenal, although their relative positions differ. As I have shown, the zone comparable to the zona glomerulosa is found at the caudal pole of the gland, that comparable to the zona reticularis forms the outermost cortical layer, whilst the zona fasciculata, generally the best-developed zone of the cortex in the Eutheria, is poorly developed and forms the innermost layer of the cortex.

The chromaffin tissue appears similar to that found in the Eutheria except that in the cytoplasm of these cells there are found vacuoles from which it is probable that lipoids have been dissolved during the fixing process. Such lipoids are not found in the medulla of the Eutheria.

One of the most interesting features of the suprarenal of the Monotreme is, however, the incomplete surrounding of the medulla by the cortex and the presence of a small ganglion mass lying close to or embedded in the medullary tissue. According to Vincent (17), in the suprarenals of young animals, the medulla is not completely surrounded by cortex, but comes to the surface at some point. Further, he says that in the suprarenal of the young rabbit "near that part of the circumference where the medulla reached the surface was a sympathetic ganglion outside the capsule of the organ." This similarity between the developing suprarenal of the young rabbit and that of the adult Monotreme is suggestive in that perhaps here we have a definite step in the evolution of the Eutherian suprarenal.

Summary

1. The suprarenal of *Echidna* is ovalish in form, and consists of a well-differentiated cortex and medulla. The cortex lies at the cranial pole of the gland and forms an incomplete covering to the medullary tissue. This latter tissue comes to the surface at the caudal end of the gland and constitutes the hilum where the blood vessels enter and leave the gland.

2. The cortex, situated at the cranial end of the gland, consists of two zones, an outer reticular zone and an inner or zona fasciculata. Between the zona fasciculata and the medulla is found a narrow reticular zone which becomes continuous with the outer zona reticularis in the caudal region. This reticular zone spreads backwards over the medulla for some distance, but caudally it is replaced by a mass of cells, the zona glomerulosa. The cells of this zone spread cranially over the zona reticularis for a short distance, separating it from the capsule.

3. The zona reticularis consists of a network of anastomosing cellular strands, in the meshes of which there are wide blood capillaries, whilst the zona fasciculata consists of more regular coarser strands between which capillaries run parallel to each other. The zona glomerulosa consists of groups of columnar cells, separated from each other by connective tissue septa. These septa in the region of the hilum often completely replace the glandular tissue.

4. The cortex receives its blood supply from the capillaries of the capsule and also from arterioles which run direct from the hilum to the cortex and there break up into capillaries in the connective tissue septa. From these the blood passes either into the cortico-medullary sinuses or into the large blood channel which runs from the cortex through the medulla to the hilum, and there gives rise to the veins.

5. The medulla forms the caudal part of the gland and is separated from the cortical tissue by a layer of connective tissue and irregular blood sinuses. It consists of groups of large granular cells with numerous large vacuoles in their cytoplasm. Intermixed with these cells are found prolongations of cortical tissue.

6. The medulla is supplied by blood vessels from the hilum which ramify among the cells and are collected either into the cortico-medullary sinuses or into the main venous channel.

7. A small ganglion composed of multipolar cells is found in the region of the hilum, often embedded in the medullary tissue. Processes from these cells ramify among the medullary cells.

This work was carried out in the Department of Histology and Embryology, under the direction of Prof. J. P. Hill, F.R.S., to whom I wish to express my thanks for permission to use the material from his collection and for his help and guidance throughout the investigation. I am indebted to Dr D. Krauz for the original drawings of text-figs. 4, 5 and 6; to Mr A. K. Maxwell for retouching the microphotographs from which Plate III, figs. 7 and 11 were made, and to Mr F. J. Pittock for making these microphotographs.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Low-power drawing of Malpighian corpuscle (*M.c.*). Mag. × 93. Mallory's connective-tissue stain. Note the Malpighian corpuscle is surrounded by a capsule (*cap.M.*) formed of reticular fibres (*ret.f.*) inside which is seen the peripheral blood-space (*bl.s.*), the blood from which is drained into the splenic pulp by means of fine capillaries (*c.*). Surrounding the Malpighian corpuscle (*M.c.*) is seen the splenic pulp (*sp.p.*) containing a small venous sinus (*v.s.*).

Fig. 2. Low-power drawing of Malpighian corpuscle (*M.c.*) to show central artery (*c.a.*). Mag. × 135. Mallory's connective tissue stain. Note the central artery (*c.a.*), surrounded by connective tissue (*c.t.*) and the blood space (*bl.s.*) at the periphery of the corpuscle, just inside the capsule (*cap.M.*). The central artery (*c.a.*) can also be seen, lying in the splenic pulp (*sp.p.*) before it enters the corpuscle (*M.c.*).

Plate II

Fig. 3. High-power drawing of trabecula of *Echidna* spleen. Mag. × 297. Ehr. haem. and Pasini. The trabecula (*T.*) consists chiefly of smooth muscle (*s.m.*) and a small quantity of connective tissue (*c.t.*). On either side of the trabecula is seen the syncytial network of reticulo-endothelial cells (*ret.c.*). Two large reticular cells (*ret.c.*) are seen intermixed with red blood corpuscles (*r.bl.*) at the right lower corner of the figure.

Fig. 4. High-power drawing of ellipsoid (*el.*). Mag. × 283. Ehr. haem. and Pasini. The terminal artery (*t.a.*) has divided into two penicillate arterioles (*p.a.*) which can be seen surrounded by a layer of reticular cells (*ret.c.*) and fibres (*ret.f.*) which constitute the ellipsoid (*el.*). The penicillate arterioles (*p.a.*) are lined by a layer of endothelial cells (*end.c.*) whose nuclei project into the lumen (*l.*), which at the funnel-shaped opening (*f.*) become flattened and not clearly distinguishable from the reticular cells (*ret.c.*).

Fig. 5. Low-power drawing of a venous sinus. Mag. × 73. Mann's methyl-blue-eosin stain. Note the large venous sinus (*v.s.*), containing blood (*bl.*) into which a number of small venous capillaries (*v.c.*) open from all sides, draining the blood from the surrounding splenic pulp (*sp.p.*). A part of the trabecula (*T.*) is seen on the left of the venous sinus (*v.s.*). Below the trabecula (*T.*) there is another small venous sinus (*v.s.*).

Fig. 6. High-power drawing of the granular cells (*gr.c.*) found in the pear-shaped enlargement of the dorsal limb of the spleen. Mag. × 439. Ehr. haem. and Dominici. Note the granular cells (*gr.c.*) lying intermixed with the reticular cells (*ret.c.*), lymphocytes (*lym.*), red blood cells (*r.bl.*) and reticular fibres (*ret.f.*) of the pulp. Compare the pycnotic nuclei (*p.nuc.*) of these cells with those of the cells of the surrounding tissue.

PLATE III

Fig. 7. Medium-power microphotograph of the cortico-medullary portion of the suprarenal. *Echidna.* Mag. × 56. Note the medulla (*chr.c.*) is separated from the cortex by irregular blood sinuses (*bl.s.*) and layers of connective tissue (*c.t.*). The layer of cortical cells (*z.r.*2) surrounding the medulla is darkly stained and contains wide capillaries. At (*j.*) it becomes continuous with the zona reticularis (*z.r.*1). The inner zone or zona fasciculata (*z.f.*) stains paler and the cells are arranged in coarse parallel strands.

Fig. 8. High-power microphotograph of the cortex, about the middle of the suprarenal. *Echidna.* Mag. × 90. Note the outer zona glomerulosa (*z.g.*) composed of oval groups of columnar cells separated by irregular anastomosing connective tissue septa, and the second layer or zona reticularis (*z.r.*1) which forms the outer layer of the cranial part of the cortex. This zone consists of an anastomosing network of cellular strands in the meshes of which are wide capillaries (*c.*). The innermost layer, zona fasciculata (*z.f.*) consists of paler staining cells arranged in coarse regular strands separated by fine reticulum.

Fig. 9. High-power microphotograph of the zona reticularis of the cortex. *Echidna.* Mag. × 180. Note the anastomosing strands of cells which form a wide meshed network, the spaces of which are occupied by large blood capillaries (*c.*). The cells are not sharply outlined from each other and their nuclei (*nuc.*) are small and spherical whilst their cytoplasm is finely vacuolated.

Fig. 10. High-power microphotograph of the zona fasciculata (*z.f.*) of the suprarenal. *Echidna.* Mag. × 180. Note the coarse regular strands of cells which are separated from each other by reticular fibres (*ret.f.*) and small capillaries. Portions of zona reticularis (*z.r.*1, *z.r.*2) are seen at the top and bottom of the figure respectively.

Fig. 11. High-power microphotograph of the zona glomerulosa (*z.g.*) of the suprarenal. *Echidna.* Mag. × 180. Note the arrangement of the cells in irregular rounded groups. These are separated from each other by anastomosing connective tissue septa (*c.t.*) derived from the capsule (*cap.*). The cells are columnar in form and their nuclei are oval or spherical. The cytoplasm contains numerous coarse vacuoles.

LIST OF ABBREVIATIONS

A.	Artery.
a.	Arteriole.
a.cap.	Arteriole to the capsule.
a.chr.c.	Anteriole to the medulla.
a.cor.	Arteriole to the cortex.
a.cor.t.	Arteriole derived from the cortical branch which runs along cortical trabecula.
a.z.f.	Arteriole to zona fascicularis.
a.z.g.	Arteriole to zona glomerulosa.
*a.z.r.*1	Arteriole to zona reticulata.
b.	Body of the spleen.
bl.	Blood.
bl.s.	Blood sinus.
c.	Capillary.
c.a.	Central artery.

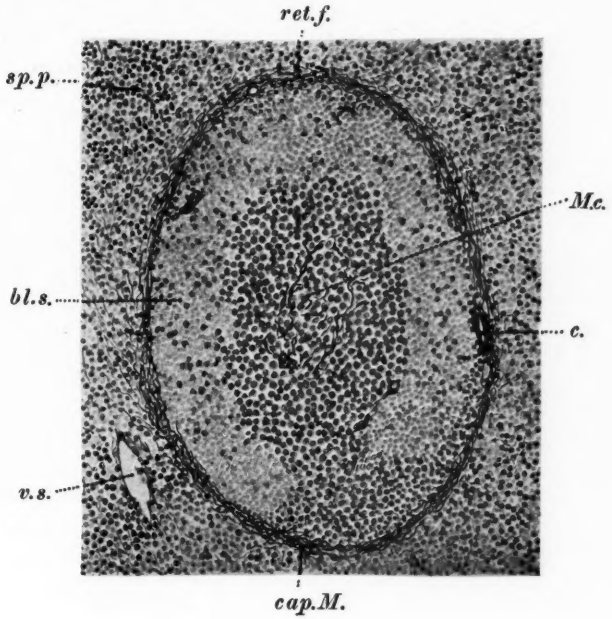

Fig. 1.

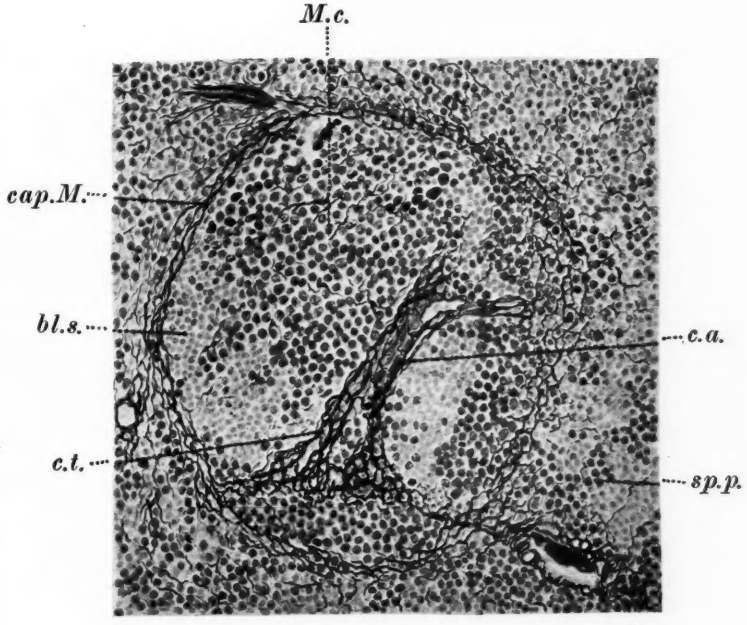

Fig. 2.

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T.
bl.
v.s.

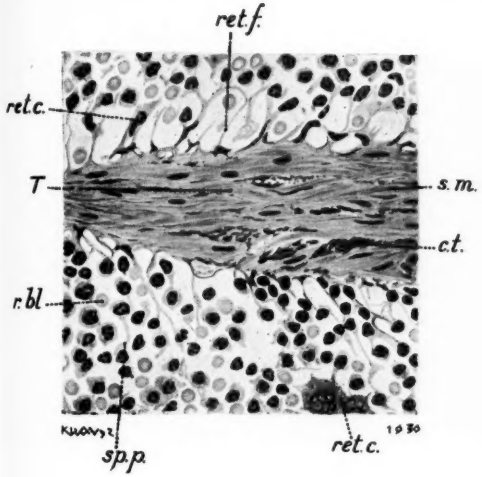

Fig. 3.

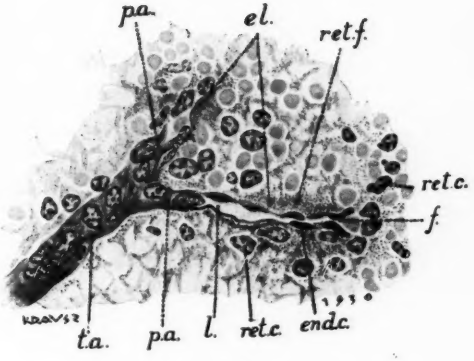

Fig. 4.

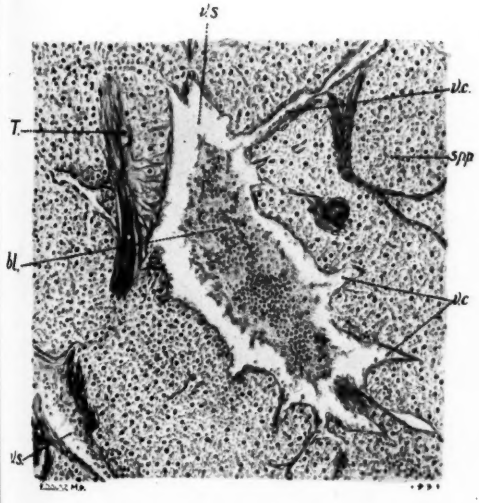

Fig. 5.

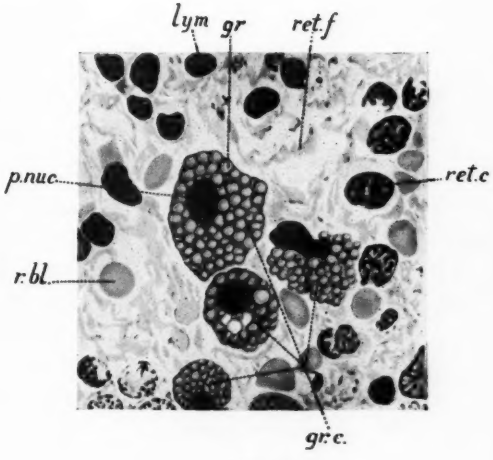

Fig. 6.

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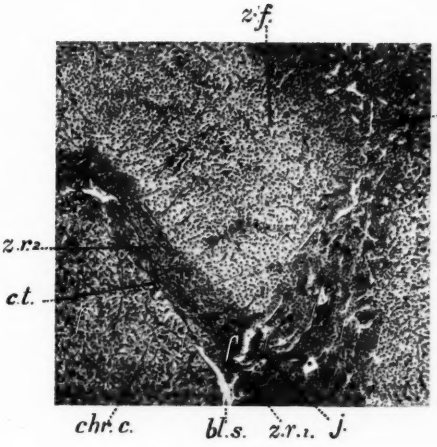

Fig. 7.

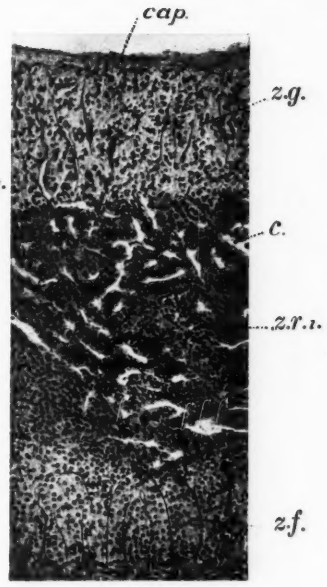

Fig. 8.

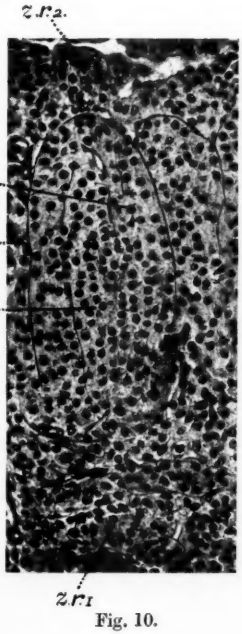

Fig. 10.

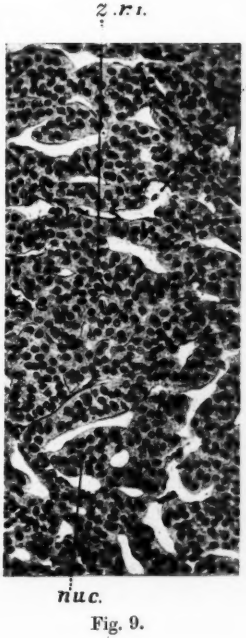

Fig. 9.

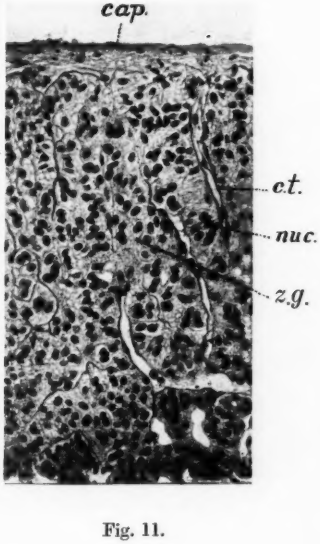

Fig. 11.

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cap.	Capsule.
cap.M.	Capsule of the Malpighian corpuscle.
cap.pl.	Capillary plexus in the capsule.
chr.c.	Medulla.
chr.c.pl.	Capillary plexus of the medulla.
c.pt.	Conical area of cells at the cranial end of the suprarena.
c.t.	Connective tissue.
c.t.	Connective tissue septum.
c.v.ch.	Central venous channel.
d.enl.	Enlargement of the dorsal process.
d.pr.	Dorsal-process of spleen.
el.	Ellipsoid.
end.c.	Endothelial cells.
f.	Funnel-like opening of the penicillate arteriole.
g.n.	Ganglion.
gr.c.	Granular cells.
h.	Hilum.
h.s.	Hilar sheath.
j.	Junction of the outer zone or zona reticularis with the deepest zone lying adjacent to the medulla.
l.	Lumen.
l.st.	Stalk of lymphoid tissue.
lym.	Lymphocyte.
M.c.	Malphigian corpuscle.
n.c.	Nerve cells.
n.f.	Nerve fibres.
nuc.	Nucleus.
p.a.	Penicillate arteriole.
*p.a.*1	Penicillate arteriole which runs into the venous sinus directly.
p.nuc.	Pycnotic nucleus.
r.bl.	Red blood cells.
ret.c.	Reticular cells.
ret.f.	Reticular fibres.
s.m.	Smooth muscle.
sp.p.	Splenic pulp.
st.	Stomata.
T.	Trabecula.
t.a.	Terminal arteriole.
t.ans.	Transverse anastomosis.
v.	Vein.
v.c.	Venous capillaries.
v.pr.	Ventral process.
v.s.	Venous sinus.
z.f.	Zona fasciculata.
z.f.pl.	Capillary plexus in zona fasciculata.
z.g.	Zona glomerulosa.
z.g.pl.	Capillary plexus in zona glomerulosa.
*z.r.*1	Zona reticularis.
*z.r.*1*.pl.*	Capillary plexus in the zona reticularis.
*z.r.*2	Zona reticularis, adjacent to medulla.
*z.r.*2*.pl.*	Capillary plexus in the zona reticularis adjacent to medulla.
*z.r.*1	Lamina of zona reticularis in the caudal part.

CONCERNING THE NASAL APERTURES, THE LACHRYMAL CANAL AND THE BUCCO-PHARYNGEAL UPPER LIP

By EDWARD PHELPS ALLIS, Jr.

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His (1892) in his work on "Die Entwickelung der menschlichen und thierischer Physiognomien," came to the conclusion that the oral and aboral nasal apertures were homologous throughout the vertebrate series, and to explain the position of the apertures on the ventral surface of the snout in embryos of the Teleostomi and on its dorsal surface in adults, he assumed that the apertures had migrated forward, upward and backward over the anterior end of the snout, thus retaining their oralo-aboral relations to each other but reversing their antero-posterior ones. This is, I believe, the present generally accepted opinion (Peter, 1901), but certain authors have contended that the pits pass upward over the lateral edges of the snout and that the apertures retain their antero-posterior relations to each other, their oralo-aboral ones being reversed. The development of the bucco-pharyngeal upper lip has a most important influence in determining the ultimate positions and relations of these apertures and has not heretofore been sufficiently taken into consideration.

The nasal plate first appears as a thickening of the entire ectoderm when the ectoderm is single-layered at this stage of development, but of its deeper layer only when it is two-layered. It lies on the morphologically lateral or ventro-lateral surface of the head, between the eyeball and the neuropore, but it may be presented ventrally, ventro-laterally or even ventro-latero-posteriorly, because of cranial flexure or the general downward curvature of the anterior end of the embryo. This plate soon sinks somewhat into the underlying tissue, the external layer of the ectoderm, when present, breaking down. The primitive nasal pit thus arises, and its external opening may be called the primitive nasal aperture. The hypophysis develops in the ectoderm that lies postero-ventral to the nasal pits, between them and the dorso-anterior edge of the bucco-pharyngeal plate, either in connection with Rathke's pocket or as a solid ingrowth either of the entire single-layered ectoderm or the deeper layer only of a two-layered ectoderm. Rathke's pocket and this solid ingrowth correspond topographically and will both be hereafter referred to as the hypophysis, the canal or cord that later extends forward from it to the external surface and that is developed in relation to the forward growth of the bucco-pharyngeal upper lip being called the hypophysial canal or cord instead of, as in one of my earlier works (Allis, 1931), the pre-hypophysial portion of the naso-hypophysial cana

or cord. The term naso-hypophysial canal is properly applicable only to the conditions in the Cyclostomata, for in no other vertebrate does this canal or cord come into direct connection with the nasal sac.

The snout of all vertebrates is either a process of cranial origin (His, 1892) into which the trabeculae have become incorporated (Allis, 1923), or such a trabeculo-cranial snout to which there has been added what Stensiö (1927) has called in *Petromyzon* a visceral component, the latter component being furnished by the forward growth of the bucco-pharyngeal upper lip. This latter lip is formed by the growth forward of the tissue that lies, in early embryos, between the ventro-posterior edge of the hypophysis and the dorso-anterior edge of the bucco-pharyngeal plate, but its development has not been fully described in any vertebrate I know of except *Petromyzon*. In the latter fish it is shown in median sagittal section by both v. Kupffer (1894) and de Beer (1923) lined both dorsally and ventrally by ectoderm, and the first impression one gets from the descriptions and figures is that the lip grows progressively forward beneath the overlying portion of the head and fuses with it excepting along the median line, thus giving origin to the naso-hypophysial canal. This is, however, not strictly correct, for when the lip in its forward growth reaches the oral edge of the nasal pit, both it and the tissue that lies between that edge of the pit and the dorso-anterior edge of the hypophysis grow forward together at the same rate of growth, the short canal already formed thus simply being prolonged and no new section added to it. As a result of this, the entire ectodermal surface in which the nasal pit lies is pushed upward and forward until it forms the anterior portion of the dorsal surface of the trabeculo-cranial component of the snout. During this process the edges of the nasal pit do not change their respective relations to the upper edge of the mouth, for the ectoderm in which the pit lies simply swings forward and upward from a ventrally to a dorsally presented position.

In the Teleostomi the bucco-pharyngeal upper lip quite certainly develops in a manner that is in principle strictly similar to that in *Petromyzon*, but its development has not been fully described. De Beer (1926) in a median sagittal section of the head of a 7 mm. embryo of *Salmo* shows conditions that are in principle similar to those shown by v. Kupffer in a median sagittal section of the head of a 5-day old embryo of *Petromyzon*. The bucco-pharyngeal upper lip has not yet begun to develop, and the only figure given by de Beer in which its later development is shown is a diagrammatic one said to represent the conditions in the teleostome fishes and Amphibia, and in it the "hypophysis," as de Beer employs this term, is shown arising as a solid ingrowth of the ectoderm on the dorsal surface of the snout. That part of the snout that lies antero-ventral to this ingrowth is evidently a visceral component formed by the forward growth of the bucco-pharyngeal upper lip. The lip must therefore here develop, in principle, as it does in *Petromyzon*, and the two nasal pits have swung forward and upward onto the dorsal surface of the snout in exactly the same manner that the single nasal pit does in *Petromyzon*; and whether in this

process the pits have the appearance of passing over the anterior or the lateral edges of the snout would not affect the relations of their edges to the eyeballs and the upper edge of the mouth. They do not, in fact, pass over either of the edges of the definitive snout, and in so far as their relations to this snout are concerned, they might be said to have passed upward through it. If each primitive aperture passed upward over the lateral edge of the snout, as Gawrilenko (1910) considers it does in *Salmo*, the olfactory nerve would necessarily have been twisted through something considerably more than 90°, and so far as I know there is not the slightest indication that this has taken place.

According to Dohrn's (1881) and Lundborg's (1894) descriptions of *Hippocampus* and *Salmo*, the bucco-pharyngeal upper lip of these fishes develops at such an early age that the hypophysial cord has the appearance of developing ontogenetically before the hypophysis, the latter arising from the dorsal wall of the deeper end of the cord, this wall corresponding to a median line along the ventrally presented surface of the anterior end of the head of early embryos of *Petromyzon*. The hypophysis in these fishes may acquire a secondary connection with the ectodermal lining of the roof of the buccal cavity. In *Amia* and *Acipenser* the same reversal in the order of the ontogenetic development of the hypophysis and hypophysial cord takes place according to the figures and descriptions of v. Kupffer (1893), de Beer (1923) and Holmgren (1931), but this does not in any way affect in principle the manner in which the nasal pits have shifted from the ventral to the dorsal surface of the snout.

In *Polypterus* the conditions are probably as they are in the Teleostomi, for although the hypophysial canal opens definitely on the dorsal surface of the buccal cavity, the nasal apertures of either side both lie on the dorsal surface of the snout in typical teleostean position.

In the Elasmobranchii and Dipnoi the conditions differ markedly from those above described in that the bucco-pharyngeal upper lip does not grow far forward and that the snout is consequently of the trabeculo-cranial type. The nasal pits accordingly here retain their embryonic positions and lie on the ventrally or ventro-laterally presented surfaces of the snout.

The nasal pits of fishes are thus seen to lie always in trabeculo-cranial tissue, and it seems quite certain that their lateral and mesial edges are homologous in all of the gnathostome fishes. In the Teleostomi, Crossopterygii and Dipnoi, these edges of each pit grow toward each other and meet and fuse above the pit. This cuts the primitive nasal aperture into two parts which form the definitive nasal apertures separated from each other by the so-called nasal bridge. These apertures both lie in trabeculo-cranial tissue and never, so far as I know, ever traverse or become surrounded by the visceral tissue of the snout. When the anterior nasal passage is to be prolonged forward beyond the edge of the trabeculo-cranial component of the snout, a tubule is developed which projects forward above the visceral component without entering it at any point.

In the Elasmobranchii the two edges of each nasal pit grow toward each other, as they do in the Teleostomi, Crossopterygii and Dipnoi, but they do not

coalesce, simply overlapping and resting one upon the other. An incomplete nasal bridge is thus formed and the primitive nasal aperture is incompletely separated into two, the bridge and apertures nevertheless quite certainly being the strict homologues of those in the Teleostomi, Crossopterygii and Dipnoi. In certain of the Plagiostomi, the oral edge of the oral nasal aperture is connected with the anterior edge of the buccal cavity by a naso-buccal groove which is overlapped and covered externally by the lateral portion of the naso-frontal process. If the lateral edge of this process were to fuse with the lateral edge of the groove, a canal would be formed which would markedly resemble in topographical position the choanal canal of higher vertebrates. The naso-buccal bridge so formed could not, however, be the homologue of the nasal bridge of the Teleostomi, for that bridge is represented in the incompletely formed nasal bridge of the Plagiostomi. If, however, the two bridges of the latter fishes were to fuse with each other, the so-formed single bridge would include the bridge of the Teleostomi (Allis, 1919).

The Reptilia may now be considered, for the conditions here are less complicated than those in the Amphibia. In these vertebrates the hypophysis develops, as is well known, in relation to a Rathke's pocket, and the hypophysial canal or cord has its attachment to the ectoderm on the dorsal surface of the buccal cavity. The bucco-pharyngeal upper lip accordingly does not extend far forward and the snout is of the trabeculo-cranial type. The nasal pit of either side lies, when it first appears, on what later will be the ventral or ventro-lateral surface of the base of the snout, and it is connected with the anterior edge of the buccal cavity by a naso-buccal groove that is apparently homologous with that in certain of the Plagiostomi. This groove, however, here becomes a closed canal by the fusion of the outer edges of its lateral and mesial walls, these walls forming the so-called lateral and mesial, or inner and outer nasal processes. This canal may be called the choanal, for it opens in the roof of the buccal cavity through the primitive choana. The external (superficial) wall of the canal may be called the choanal bridge and it forms part of the so-called primitive palate. This bridge is generally considered to be the homologue of the nasal bridge of the Teleostomi (Peter, 1901), but this implies that the choana is the homologue of the oral nasal aperture of the latter fishes, and regarding this there is some question as already suggested when considering the Plagiostomi.

In *Lacerta*, which is alone here considered, the choanal canal is said by Born (1879) to be prolonged forward soon after it becomes enclosed, the nasal sac at the same time growing posteriorly. The ectodermal tissues that enclose the choanal canal in growing forward push the external nasal aperture before them from the ventral to the dorsal surface of the snout, but apparently do not in any way affect the position of the nasal sac. An atrial chamber (Vorhoehle) then develops in the external nasal passage, between its external opening and the nasal sac, and the choanal canal opens into it. The edges of the external aperture apparently retain their oral and aboral relations to each other, but

their antero-posterior relations are reversed as in the Cyclostomata and Teleostomi. The nasal pit does not apparently shift from the ventral to the dorsal surface of the snout, but in connection with the growth posteriorly of pocket-like extensions of the sac, the external opening of the pit becomes directed dorso-posteriorly instead of ventrally or ventro-laterally.

The trabeculae fuse with each other at an early stage of development to form the median trabecula communis which is said to grow forward between and below the nasal sacs and so form the ventral edge of the septum nasi and the floors of the nasal capsules (Born, 1879; Gaupp, 1900; de Beer, 1930). A large fenestra in the floor of each capsule gives passage to the choanal canal, a large foramen in the roof of the capsule giving passage to the olfactory nerve. The dorsal part of the septum nasi apparently develops wholly independently of the trabecula communis, but later fuses with it. The conditions therefore strongly suggest that the nasal capsules have simply been crowded toward each other and have met and fused with each other dorsal to the trabecula communis instead of ventral to it, as they apparently do in certain of the Plagiostomi.

The lachrymal canal first appears as a thickening of the ectoderm along a short and narrow line in the region between the eyeball and the nose, this line becoming constricted off from the rest of the ectoderm and sinking into the underlying tissue. The primarily solid cord so formed then grows toward the nasal pit and toward the eyeball and a canal develops in it.

In the Amphibia the "hypophysis" is said by de Beer to develop as it does in the Teleostomi, and in the diagrammatic figure referred to when considering the latter fishes it is shown growing inward as a solid strand from the dorsal surface of the snout. The snout must accordingly be of the viscero-trabeculo-cranial type, and the visceral component as it grew forward must have caused the nasal pits and the primitive nasal apertures to swing upward and forward onto the dorsal surface of the snout, as they do in the Cyclostomata and Teleostomi. There is, however, in the Amphibia as in the Reptilia, a choanal canal which is not found in fishes. In the Urodela and Anura this canal develops in a solid strand of tissue which grows ventro-posteriorly from the nasal sac and is said to fuse with the dorsal surface of the fore-gut. In the Gymnophiona the strand is in large part constricted from the deeper portion of an ingrowth of the external ectoderm and has its attachment to the ectodermal, instead of to the endodermal, lining of the roof of the buccal cavity (Peter, 1901).

Comparing the conditions in these Amphibia with those in the Reptilia, it seems quite certain that there must have been primarily in both these groups a naso-buccal groove on either side of the head and that, in the Urodela and Anura, as the bucco-pharyngeal upper lip grew forward, it was so wide that it completely covered both these grooves, but in the Gymnophiona, not wide enough to completely cover them. The ectodermal tissues that line these grooves of either side were accordingly forced beneath the surface throughout their entire lengths in the Urodela and Anura, and the choanal canals when they

developed in them, are said to open, in embryos, in the roof of the fore-gut. In the Gymnophiona, where the naso-buccal grooves were only partially covered by the bucco-pharyngeal upper lip, the choanal canals open, as in the Reptilia, in the ectodermal instead of in the endodermal portion of the roof of the buccal cavity. This is, however, obscured and confused in the ontogenetic development.

The external nasal aperture of either side is thus at this stage of development, what remains of the primitive aperture after the choanal canal has been cut out of the oral prolongation of the oral edge of the aperture and a lachrymal canal is then cut out of a similar prolongation of the aboral edge of the aperture. This is clearly shown in *Rana*, where, according to Born (1876), the lachrymal canal begins as a thickening of the external ectoderm which grows progressively posteriorly from the aboral edge of the aperture toward the eye and becomes constricted off from the ectoderm as a solid strand in which a canal later develops. That end of the canal that extends toward the eyeball then becomes separated into two branches by what must be in principle a process of dichotomous subdivision similar to that which takes place in the subdivision of the pores of the latero-sensory canals in *Amia* (Allis, 1889), these two branches of the canal opening on the inner surface of the eyelid by apertures one lying posterior to the other.

A choanal canal is probably present in all of the Amphibia and Amniota, and in most of them there is also a lachrymal canal. There are therefore in these vertebrates three canals leading into the nasal pit, while in the Cyclostomata there is but one, and in most other fishes two. One of the three canals of the Amphibia and Amniota must therefore be absent in fishes, and if the choana were the homologue of the oral nasal aperture of fishes, as it is generally considered to be (Peter, 1901), it would be the lachrymal that is wanting.

The lachrymal canal is functionally simply a drain pipe that carries away fluids that accumulate in the pocket-like spaces between the eyelids and the external surface of the eyeball. These fluids are protective in their action, cleansing and moistening the exposed conjunctival tissues, and they escape through the lachrymal canal either into the nasal sac and secondarily from it into the buccal cavity, or, as in the snake, directly from the eye into the latter cavity. In fishes there is no functional need of a lachrymal canal, for the exposed surface of the eyeball is kept cleansed and moistened by the water in which the fish lives. There is accordingly no lachrymal canal in any of them, so far as I can find described, and according to Kingsley (1917) there are also no lachrymal glands. In the Urodela the lachrymal canal appears in the Salamandridae and is there associated with movable eyelids and life on land. In the Amphiumidae, Proteidae and Sirenidae, which live in the water, the eyelids are fixed, as they are in fishes, and I can find no mention of a lachrymal canal in any of them. In most of the Anura and Amniota the canal is present and subject to but slight variation. In the crocodiles which live in the water, there are said to be lachrymal canals but no lachrymal glands, while in the

turtles, which also live in the water, there are no canals but largely developed glands.

The wide distribution of the lachrymal canal among land-living vertebrates evidently shows that it must have been promptly acquired as soon as their ancestors left the water, and as there is no lachrymal in fishes, this canal of land-living vertebrates must necessarily have either been developed independently and expressly for the function it fulfils, or some pre-existing canal that subserved a different function must have been utilised for the purpose. The ontogenetic development of the canal in *Rana* is evidently unfavourable to the first of these two assumptions, for if the canal had been developed especially for the function it fulfils it would have grown progressively from the eye toward the nasal sac, instead of, as it actually does, from the external nasal aperture toward the eye. Furthermore, the conditions in certain of the Plagiostomi are unfavourable to the assumption that the choana is the homologue of the oral nasal aperture of fishes, as already explained, and what seems still more important, there is in certain fishes, and particularly in *Polypterus* and *Amia*, a canal so placed that it could readily have been utilised to form the lachrymal.

The premaxillary of *Polypterus* is formed by the fusion of a tooth-bearing bone, that corresponds to the premaxillary of *Amia*, with the latero-sensory canal bones that correspond to the antorbital and one-half of the median ethmoid of the latter fish; the maxillary being formed by a tooth-bearing component that contains some part of the maxillary of *Amia* fused with certain of the infra-orbital latero-sensory canal bones of the latter fish (Allis, 1900 *a*). The lachrymal is an independent bone in both these fishes and may be referred to as the piscine lachrymal. If this lachrymal of *Polypterus* were to fuse with the maxillary, a process of the latter bone would be formed corresponding to the posterior portion of the frontal process of the superior maxillary of Man and which is considered to be of maxillary origin; the antorbital process of the premaxillary of *Polypterus* corresponding to the anterior portion of the frontal process of Man which is considered to be of premaxillary origin (Allis, 1900 *b*). The foramen infraorbitale lies between these two parts of the process of Man and the ramus maxillaris trigemini of *Polypterus* issues from the orbit in the angular space between the lachrymal and the antorbital process of the premaxillary.

The dorso-posterior end of the antorbital process of the premaxillary of *Polypterus* is considerably expanded and has a relatively long and straight dorsal edge which is presented dorso-mesially and extends from the posterior edge of the external opening of the nasal pit to the anterior edge of the external opening of the orbit. The dorsal edge of the process is thin and passes beneath the nasal bone and the anterior end of the frontal, the anterior portion of this part of the process lying directly upon the dorso-lateral surface of the nasal capsule and its posterior portion lying upon the dorsal surface of the ectethmoid. This latter bone, which is the prefrontal of Traquair's (1871) descriptions, is apparently of endosteal origin and, extending from the nasal

capsule to the orbit, forms part of the bounding wall of the former and part of the anterior wall of the orbit. Immediately posterior to it there were, in one of the specimens particularly examined by me (Allis, 1922), two small scale-like bones which were apparently of endosteal origin, but in a position that is topographically markedly similar to that of the lachrymal of Man. The piscine lachrymal forms the antero-ventral bounding edge of the orbit and is in contact with the antero-laterally presented edges of the two little ectethmoidal plates above referred to. The posterior nasal passage has been prolonged posteriorly until its external aperture lies close to the anterior edge of the eye, the canal leading from it to the nasal sac passing across the external surface of the thin dorsal edge of the antorbital process of the premaxillary close to the lateral edge of the nasal bone and then over the posterior edge of the fenestra nasalis.

In *Amia calva* the conditions are similar to those in *Polypterus*, but differ somewhat in detail. The latero-sensory canal bones have not fused with the maxillary and premaxillary. The antorbital bone lies against the lateral edge of the nasal, but does not extend dorsally beneath it, its dorso-posterior end lying in the transverse plane of the hind edge of the nasal and leaving a considerable interval between itself and the anterior edge of the orbit. Dorsally this interval is bounded by the lateral edge of the anterior end of the frontal, and ventrally by the dorsal edge of the piscine lachrymal, a little rectangular space depressed somewhat beneath the outer surface of the dermal cuirass that covers the head thus being left between these several bones. The floor of this space is formed by the ectethmoid which is apparently of endosteal origin with a dermal component added to its dorsal surface. This little space is in the fresh specimen filled with tough connective tissue, and the posterior nasal aperture lies at about the middle of its length and hence not far from the anterior edge of the orbit. The canal leading from the aperture to the nasal sac passes through a foramen in the angle between the adjoining edges of the nasal and antorbital bones, thus lying external to the ectethmoid and morphologically internal to both the antorbital and piscine lachrymal.

The posterior nasal apertures and related canals in these two fishes could thus with but slight changes in the region have been readily utilised to form the lachrymal canal of higher vertebrates.

CONCLUSIONS

The snout of vertebrates is either of trabeculo-cranial or viscero-trabeculo-cranial origin, the visceral component of the latter being furnished by the forward growth of the bucco-pharyngeal upper lip and forming the anterior end of the snout and an apparently variable part of its ventral surface.

In the Elasmobranchii, Dipnoi and Amniota the snout is of the trabeculo-cranial type and in the Cyclostomata, Teleostomi and Amphibia and probably also in the Crossopterygii of the viscero-trabeculo-cranial type.

The nasal pits always lie in the trabeculo-cranial tissue, on the ventral or

ventro-lateral surface of the snout when it is of the trabeculo-cranial type, but on its dorsal surface when it is of the viscero-trabeculo-cranial type.

The choana of the Amphibia and Amniota is apparently the homologue of the oral opening of the naso-buccal groove of certain of the Plagiostomi, and hence is not the homologue of either of the nasal apertures of fishes.

The lachrymal canal of the Amphibia and Amniota is probably represented in fishes, in the aboral nasal aperture and the canal leading from it to the nasal pit.

The external nasal aperture of the Amphibia and Amniota corresponds to the oral nasal aperture of fishes.

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THE PRE-ORAL GUT, THE BUCCAL CAVITY AND THE BUCCO-PHARYNGEAL OPENING IN *CERATODUS*

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Gregory (1905) in a work on "Die Entwickelung der Kopfhoehlen und des Kopfmesoderms bei *Ceratodus forsteri*," published in the fourth volume of the *Denkschriften der medicinisch-naturwissenschaftlichen Gesellschaft zu Jena*, came to the conclusion that there was in early embryos of this fish a pre-oral gut strictly comparable to that described by v. Kupffer (1894) in embryos of *Petromyzon*. Greil (1908, 1913), however, in an extensive work on the development of this same fish published in that same volume of the *Denkschriften*, concluded that the mouth opening was definitely terminal and that there was accordingly at no period of ontogenetic development a morphologically pre-oral portion of the gut. The conditions in this region of the head of this fish, as described by Greil himself, do not however seem favourable to this view.

In early embryos of *Ceratodus*, the archenteric cavity is said to extend forward somewhat beyond the anterior end of the neural plate, the dorsal wall of the cavity presenting in median sagittal section a strongly and evenly curved line. The median point of the anterior end of the neural plate forms the ventral boundary of the future neuropore, and beginning immediately posterior to it, in slightly older embryos, the middle portion of the neural plate sinks inward somewhat below the level of its lateral edges, this being particularly marked at its anterior end. A neural groove is thus developed the anterior end of which curves somewhat abruptly outward and forms the anterior wall of the embryonic brain. The infundibulum lies in the floor of the groove slightly posterior to its anterior end.

The sinking inward of the anterior end of the neural plate depresses the underlying part of the archenteric cavity and the mid-dorsal line of that cavity, as shown in median sagittal section, curves evenly downward and forward until it reaches the anterior surface of the embryonic brain. There it curves somewhat abruptly outward closely against the anterior surface of the brain and extends almost if not quite to the neuropore. Slightly anterior to this, there is a slight depression on the outer surface of the ectoderm which marks the beginning of the head-fold, and directly internal to it a corresponding depression in the external wall of the archenteric cavity. This latter depression is called by Greil the "Grenzfalte" and it separates the anterior end of the archenteric cavity into two parts, a dorsal one which is said to form the anterior end of the alimentary canal, and a ventral one which forms the so-

called ventral gut in relation to which the liver and certain other organs develop. In later stages this ventral gut arises from the alimentary canal posterior to the branchial chamber. The short ventral surface of the so-called anterior end of the alimentary canal of the early embryos here under consideration must accordingly represent the floor of the entire branchial and prebranchial portions of the alimentary canal.

In a slightly older embryo the Grenzfalte and head-fold have slightly deepened and the floor of the so-called anterior end of the alimentary canal has been correspondingly lengthened. The so-called anterior end of the alimentary canal now forms a short conical process which projects outward from the antero-ventral end of the dorsal wall of that part of the archenteric cavity that is later called the fore-gut.

In the next following stages the brain has increased in size and length, and this has gradually crowded the process-like anterior end of the alimentary canal out of the space between the anterior surface of the embryonic brain and the dorsal wall of the head-fold, the latter wall now forming the ectodermal covering of this part of the brain. The process however still persists as such in an embryo of Stage 30, but at Stage 31 it has wholly disappeared. Just what happens to it is not clear. Its walls do not break down and become either dispersed or absorbed in the dorsal wall of the fore-gut, as they do in *Petromyzon*, *Amia*, *Salmo* and the Plagiostomi (Allis, 1931); and it seems exceedingly probable that the walls of the process simply spread apart somewhat and form the antero-ventral corner of the fore-gut,the cavity of the process being added to the cavity of that gut.

In an embryo of Stage 25, the hyomandibular visceral pouch has just made its appearance and at Stage 27 it has broken through the overlying sheet of mesoderm. It is said to lie nearly parallel to the notochord and is shown extending from above downward and forward and directed toward that part of the ventral wall of the anterior end of the alimentary canal that will later become the floor of the branchial chamber. That part of the covering sheet of mesoderm that lies immediately dorso-anterior to the pouch, and hence morphologically anterior to it, represents the mandibular visceral arch, that arch extending dorso-posteriorly beyond the dorsal end of the pouch and ending in the region lateral to the anterior end of the notochord. The distance along the dorsal wall of the alimentary canal from the dorsal end of the mandibular arch to the hind edge of the dorsal surface of the process-like anterior end of the canal is thus relatively long compared with the length, at this stage, of the floor of the corresponding part of the canal; and it is this that gives to the visceral arches their inclined position from above downward and forward, which differs so markedly from that in the Plagiostomi, where the arches incline from above downward and backward. In the Teleostomi, *Urodela* and *Anura*, the arches also incline from above downward and forward, as they do in *Ceratodus*, but I have not attempted to trace their development and that of the related portion of the alimentary canal. It may however be

noted that in all of these vertebrates the hypophysis develops as a solid ingrowth and not in relation to a Rathke's pocket, as it does in the Plagiostomi and probably in all of the Amniota.

In embryos of *Ceratodus* older than Stage 27, just above considered, the Grenzfalte and head-fold both deepen progressively and the floor of the branchial chamber is correspondingly lengthened. This causes the ventral end of the hyomandibular visceral pouch of either side to swing downward and backward and this is accompanied by a corresponding swinging downward and backward of the related portion of the mandibular arch, that portion of the latter arch that lies dorsal to the pouch retaining its primitive position nearly parallel to the notochord. There is therefore a marked bend in the arch, the part that lies ventral to the bend forming the epal and ceratal elements of the arch, as Greil has stated, while the dorsal portion quite certainly forms the pharyngeal element and later the polar cartilage although this is not so stated by Greil and cannot be definitely traced in his figures and descriptions.

The hypophysis is first mentioned in an embryo of Stage 31, where it appears as a thickening of the entire ectoderm in the region immediately posterior to the antero-ventral corner of the embryonic brain. The ectoderm is here single-layered and corresponds to the deeper layer only of the ectoderm in closely adjacent regions, the outer layer of the ectoderm of these latter regions later closing in and completely covering the external surface of the hypophysis, this suggesting the closing of the outer end of Rathke's pocket during certain stages in the development of the hypophysis in the Plagiostomi. The hypophysis then grows inward as a plate-like process between the floor of the brain and the dorsal wall of the alimentary canal and apparently lies along the external surface of what remains of the dorsal wall of the process-like anterior end of the alimentary canal. Slightly posterior to the line of origin of the hypophysis a thick bucco-pharyngeal plate develops and extends posteriorly to the ventral ends of the mandibular arches, the plate later breaking through to form the bucco-pharyngeal (oral) opening. In a median sagittal section of an embryo of Stage 42½, Semon (1901 *a*) shows the bucco-pharyngeal upper lip forming a low and rounded transverse ridge on the dorsal surface of what will later be the buccal cavity, but it soon flattens out, as shown in Greil's several figures, and the lip does not undergo further development.

Immediately anterior to the mandibular arch, Greil describes (p. 1010) what he calls the premandibular visceral pouch, but as this pouch is usually referred to as the mandibular it seems best to employ that term. This pouch is said to lie in the lateral wall of the alimentary canal dorsal or postero-dorsal to the bucco-pharyngeal opening and apparently in direct connection with that opening. Immediately anterior to this pouch there is a depression in the lateral wall of the alimentary canal filled with what Greil calls free mesodermal cells, and in these cells what he calls the premandibular pterygoid process develops (p. 994). The anterior boundary of this depression is formed by what Greil calls the prepterygoid visceral pouch. It however seems simply to be

a fold in the lateral wall of the alimentary canal that forms the anterior boundary of the depression in which the cells that give origin to the pterygoid process lie.

The so-called premandibular pterygoid process above referred to is certainly some part of the cartilaginous premandibular arch, as Greil considers it to be, but it is said by him to be unimportant, inconstant and temporary. In an embryo of Stage 45, it is said to arise at the point where the anterior process of the pterygoquadrate fuses with the hind end of the trabecula, but it is not said whether the process arises from the pterygoquadrate or the trabecula. The point where these three cartilages fuse with each other lies at the anterior end of that part of the trabeculo-polar bar that forms the lateral bounding wall of the hypophysial fossa and that accordingly is, or at least contains, the polar cartilage. That the dorsal end of the pterygoquadrate fuses with this polar cartilage at its anterior end is thus in accord with my contention that the polar cartilage is the pharyngeal element of the mandibular arch. The pterygoid process is said to give support in early embryos to the postero-lateral end of a dermal plate in relation to which the vomero-palatine teeth of Greil's terminology develop. This will be again referred to when considering the buccal cavity.

Comparing the conditions in *Ceratodus* with those in the Plagiostomi, it seems quite certain that the dorsal wall of the process-like anterior end of the alimentary canal of early embryos of *Ceratodus* is the homologue of all or a part of the dorsal wall of the pre-oral gut of the Plagiostomi. The anterior end of the canal of *Ceratodus* is therefore quite certainly a pre-oral gut, as Gregory considered it to be. The bucco-pharyngeal opening of *Ceratodus* must then be the homologue of that of the Plagiostomi, and the latter opening is quite certainly a perforation, *in situ*, of the ventral wall of the alimentary canal between the ventral ends of the mandibular and premandibular arches (Allis, 1931). Greil however is not of this opinion, at least in so far as *Ceratodus* is concerned, for he considers the bucco-pharyngeal opening of this fish to be morphologically terminal, the process-like anterior end of the alimentary canal, called by him the "innere Mundbucht," having been forced downward and backward by the growing brain in such a manner that its dorsal and ventral surfaces spread apart and the process, flattened out, forms the anterior portion of the floor of what is now called the fore-gut. The bucco-pharyngeal opening thus came to lie on the ventral surface of the alimentary canal and that part of the dorsal wall of that canal that lies morphologically immediately posterior to this opening bulged forward in earlier embryos and was therefore topographically pre-oral in position, although morphologically post-oral. The tip of the canal, in thus shifting ventro-posteriorly, would necessarily have pushed before it the ventral ends of all of the anterior visceral arches, but while it does actually so push backward the ventral ends of the mandibular arches, those ends of the premandibular arches continue to be directed from above downward and forward anterior to the bucco-pharyngeal opening, this

seeming to be definitely unfavourable to Greil's view. This relation of the premandibular arches to the opening might however be accounted for by assuming that as the primarily terminal opening shifted posteriorly it passed between their ventral ends and this is practically what de Beer assumes to have taken place, in the Gnathostomata, in the latest work I know of relating to this subject.

According to the latter author (de Beer, 1931), there was primarily in all craniate vertebrates a pair of premandibular visceral arches, and but one such pair, and between this pair and the mandibular arches there was on either side a primarily functional gill cleft. The mouth opening was primarily terminal and developed as a result of the breaking through of a membrane called the velum formed by the fusion of the anterior wall of the alimentary canal with the bottom of a stomodaeal depression in the external ectoderm. In *Petromyzon* the primarily terminal stomodaeal depression is said to have become deepened by sinking inward between the premandibular arches, until it reached the mandibular visceral pouches, where it expanded laterally and obliterated these pouches. The bottom of the depression then formed the velum, which later breaks through to form the bucco-pharyngeal opening, the assumed phylogenetic development of the latter opening thus not being even indicated in ontogeny. In the gnathostome vertebrates the stomodaeal depression of *Petromyzon* is considered to have swung downward and backward between the ventral ends of the premandibular arches and to have acquired a position between those arches and the mandibular ones. The premandibular arches, which in *Petromyzon* are considered to lie in the lateral walls of the stomodaeum would then, in the gnathostomes, lie in its dorsal wall in the position occupied by the trabeculae.

In *Amphioxus* the velum is said by de Beer to lie at the anterior end of the alimentary canal and he further says that there is no pre-oral gut. The conditions in this vertebrate are therefore considered by him to resemble those assumed to have existed in primitive vertebrates, the velum being terminal in position and the homologue of that in *Petromyzon*. This is evidently not in accord with van Wijhe's (1893) contention that the velum of *Amphioxus* develops in relation to the left hyomandibular visceral cleft and it seems also not to be in accord with the development of the pre-oral portion of the head of this vertebrate.

In early embryos of *Amphioxus* the anterior end of the archenteric cavity is said by authors to become constricted off from the more posterior portion and it would therefore seem to correspond strictly to the pre-oral gut of the craniates. In the latter vertebrates, this separation of the pre-oral gut from the fore-gut and its ultimate disappearance is due to pressure upon it of the growing brain, but in *Amphioxus* the brain is separated from the canal by the anterior prolongation of the notochord, and it is the dorsal surface of the latter that is compressed by the growing brain and even slightly hollowed out (v. Kupffer, 1905, fig. 4). It would therefore seem as if, in *Amphioxus*, the

separation of the pre-oral gut from the more posterior portion must have been inherited from ancestors in which the notochord had not yet become prolonged anteriorly.

The pre-oral gut of *Amphioxus*, as thus defined, soon separates into two parts, one right and the other left, this probably being due to pressure of the notochord, as suggested by van Wijhe. The right-hand portion becomes extended forward beneath the notochord and is called by Goeppert (1902) the rostral cavity. The left-hand portion remains comparatively short, and breaking through to the exterior, forms the pre-oral pit. The external opening of this pit thus apparently lies definitely at the anterior end of the alimentary canal and is hence terminal in position. According to Goeppert, it is said by Legros to become enlarged and then to separate into three portions, one of which becomes the wheel-organ (Raederorgan) and a second one the Hatschek's groove. The third portion loses its connection with the exterior and growing inward meets and opens into the fore-gut immediately dorso-posterior to the velum, thus having to the latter membrane the topographical relations that Seessel's pocket has to the bucco-pharyngeal plate in the Plagiostomi. Van Wijhe (1893) could not trace the origin of this pocket, called by him the Schlundfortsatz, but if Legros's observations are correct it would seem to be nothing but the re-establishment of the connection of the pre-oral gut with the fore-gut after it had been constricted off from that gut. The external opening of the pre-oral gut would then be a premandibular opening or cleft that must have been present in primitive vertebrates and the wheel-organ and Hatschek's groove would have been developed from a sensory or glandular organ primarily related to that cleft and represented in the Gnathostomata by the glandular portion of the hypophysis which always develops in the ectoderm near the anterior end of the pre-oral gut (Allis, 1931).

If, as seems probable, the external opening of the pre-oral pit is a terminal opening leading into the anterior end of the alimentary canal, the larval mouth of *Amphioxus* cannot be such an opening, whether the mouth lies anterior to the mandibular arch, as de Beer apparently considers it to, or posterior to that arch, as van Wijhe maintains; and it also cannot be the homologue of the bucco-pharyngeal opening of the craniates, for that opening quite certainly lies posterior to the pre-oral gut. There is apparently no bucco-pharyngeal upper lip comparable to that in *Petromyzon*, the oral hood being formed, as it is in *Bdellostoma*, by the downgrowth of lateral longitudinal ridges. There is in fact the suggestion that if the notochord of *Bdellostoma* were to be prolonged forward to the extent it is in *Amphioxus*, certain of the features of this part of the head of *Amphioxus* might result.

Certain features of the buccal cavity of *Ceratodus* may now be considered. This cavity first appears as a slight triangular depression on the ventral surface of the head. Its anterior edge is formed by the posteriorly presented convex surface of that part of the external surface of the head that lies between the nasal pits and the hypophysis, and each lateral edge by an ectodermal ridge

that encloses the epal and ceratal elements of the mandibular arch of its side. The roof of the cavity is formed by the dorsal wall of the head-fold. The cavity is deepest at its anterior end and there extends somewhat beneath (dorsal to) the posteriorly bulging anterior surface of the head. The line of attachment of the stalk of the hypophysis lies in the deepest part of the cavity, the bucco-pharyngeal plate lying posterior to it in the roof of the cavity. In later stages the postero-ventral ends of the lateral bounding ridges of the cavity swing progressively forward, an angle which represents the angle of the gape thus being formed between the epal and ceratal elements of each ridge. These two parts of the ridge of each mandibular arch are called by Greil and Semon the Oberkieferwulst and Unterkieferwulst, but may be referred to as the pterygo-quadrate and mandibular ridges. Anterior to the pterygoquadrate ridge, and forming an anterior prolongation of it, there is in embryos of certain vertebrates a ridge that encloses the palatine process of the pterygoquadrate and that may accordingly be called the palatine ridge. This ridge forms part of what G. Haller (1923) calls in embryos of the Plagiostomi, the Oberkieferfortsatz, the other part of that process enclosing the trabecula, and as the entire process is quite certainly of premandibular origin (Allis, 1931) it may be called the premandibular process. The palatine and pterygoquadrate ridges together form what is commonly called by English authors the maxillary process, but in my earlier work, above referred to, this term, maxillary process, was employed to designate simply the Oberkieferfortsatz of Haller's descriptions.

In an embryo of *Ceratodus*, Stage 43, the cranial flexure and the transverse hypophysial furrow have been somewhat reduced. The bucco-pharyngeal opening is said by Semon (1893) to have broken through and there is no indication of either frontal or nasal processes. In the angle between the pterygoquadrate ridge and the lateral edge of the roof of the buccal cavity a flat and slightly elevated band is said by Semon to begin to extend antero-mesially on the roof of the buccal cavity and in later stages to reach and fuse in the median line with its fellow of the opposite side. He says of this band and its fellow of the opposite side (p. 44): "Ich trage naemlich einige Bedenken sie als Oberkieferfortsaetze zu bezeichnen, da man bei Fischen von einem Oberkiefer in dem Sinne des Oberkiefers der hoecheren Wirbelthiere nicht reden darf." Greil's so-called premandibular pterygoid process quite certainly develops in the basal portion of this so-called Oberkieferfortsatz but neither he nor Semon make any mention of it. A dermal bone, called by Semon (1901 *b*) the Munddachplatte, is said to develop in relation to this band and to bear teeth which he calls the vomerine and pterygo-palatine. Greil, however, calls the vomer teeth of Semon's description the premaxillary, and the pterygo-palatine the vomero-palatine. These latter teeth apparently lie posterior to the line of origin of the hypophysial ingrowth, but as the stalk of the hypophysis has already broken down and been dispersed before the teeth are developed, this cannot be definitely established from the figures and descriptions. Semon says that the Oberkieferfortsatz of this fish is the homologue of the palatine

process of the Plagiostomi, the teeth that develop in relation to it must accordingly have been considered by him to be the homologues of the teeth that form the dental arcade of the latter fish. They certainly strongly suggest that arcade, but it is to be noted that the latter arcade lies definitely anterior to the anterior edge of the bucco-pharyngeal upper lip. The teeth of *Ceratodus* are apparently the homologues of the vomero-palatine teeth of the Teleostomi and Amphibia and these latter teeth lie definitely on the ventral surface of the bucco-pharyngeal upper lip near its anterior margin, as does also the so-called maxillary tooth-bearing plate of Kiaer's (1928) descriptions of *Pteraspis*.

Greil's premaxillary teeth, called by Semon the vomerine, are represented when they first appear by two teeth, one on either side, that lie slightly anterior to the vomero-palatine teeth between the nasal pits, now converted into nasal grooves. If they are the homologues of the pre-maxillary teeth of the Teleostomi, as the name implies and as seems quite evident, they must be developed in relation to a short median section of what I have described (Allis, 1919) as the secondary upper lip; and as the nasal grooves have not yet been bridged, this secondary lip must have passed posterior to them between the grooves and the primary upper lip. The posterior nasal apertures, when developed, would then lie external to the secondary upper lip and hence could not be the homologues of the primary choanae of higher vertebrates (Allis, 1919, 1932).

De Beer, in the work already above referred to (1931), describes the early development of the trabecula in *Scyllium*, *Salmo*, *Rana* and *Amblystoma*, and comes to the conclusion that I am probably correct in my contention that this cartilage is of premandibular origin and represents the dorsal half of the bar of the arch. The ventral half of the bar is however considered to have broken down and completely disappeared, the palatine process being of mandibular origin and simply an outgrowth of the pterygoquadrate cartilage. It and the trabecula are nevertheless said to have a "community of origin," the groups of cells in which they arise being continuous with each other in early embryos and connected by a strand of similar tissue with the mandibular mesenchyme. The so-called pterygoid process of Greil's descriptions of *Ceratodus* is certainly the homologue of the palatine process of the Plagiostomi, and it is certainly premandibular in position. If then the trabecula represents the dorsal half of the premandibular bar, which de Beer is inclined to admit, the palatine process must represent some part of its ventral half.

The mandibular and maxillary branches of the nervus trigeminus are generally considered to represent the post-trematic and pre-trematic branches of a branchial nerve and they are said by de Beer to fork over the angle of the gape, that angle having "usurped the position of the former mandibular cleft." In the Plagiostomi and Teleostomi, the trunk of the nerve runs antero-laterally until it reaches, approximately, the level of the hind end of the trabecula, which represents the dorsal end of the premandibular visceral bar. There it separates into its mandibular and maxillary branches, the mandibular

branch turning postero-ventrally external to the epimandibula (pterygo-quadrate) and when it reaches the angle of the gape there turning anteriorly along the external surface of the ceratomandibula (Meckel's cartilage). The maxillary branch, after separating from the mandibular one, runs forward somewhat parallel to the trabecula, until it reaches, approximately, the level of the anterior end of the palatine process. There it sends branches forward in the direction of the anterior end of the trabecula, but in large part turns postero-ventrally in a direction somewhat parallel to the palatine process and extends toward the angle of the gape, but does not usually reach that angle and never, so far as I know, passes downward around it. These two branches of the trigeminus thus have to these several cartilages the relations of the post-trematic and pre-trematic branches of a mandibular visceral nerve, and the dorsal end of the mandibular pouch would seem to have lain considerably dorsal to the actual angle of the gape.

The nervus profundus, which is now generally considered to be the nerve of the premandibular arch, has two main branches which were formerly called the ramus ophthalmicus profundus trigemini and the portio ophthalmici profundi trigemini (Allis, 1925). The ramus ophthalmicus profundus, when present, runs forward between the superior and inferior branches of the nervus oculomotorius and then dorsal to the nervus opticus, and is distributed largely to the dorsal surface of the snout. It thus has, in its course, no direct relations whatever to the cartilaginous bar of its arch, and this is characteristic of the dorsal and not the ventral branches of the cranial nerves. The ramus ophthalmicus profundus is nevertheless generally considered to be a ventral branch that has been forced away from its primitive relations to the bar of its arch by the marked development of the eye.

RESULTS

The "innere Mundbucht" of Greil's descriptions of *Ceratodus* is a pre-oral gut strictly similar to that of *Petromyzon* and the Plagiostomi.

The bucco-pharyngeal opening is, as in *Petromyzon* and the Plagiostomi, not terminal.

The bucco-pharyngeal upper lip does not extend farther forward than the line along which the hypophysial ingrowth has its origin, that line lying on the roof of the buccal cavity.

The so-called premandibular pterygoid process of Greil's descriptions is evidently the homologue of the palatine process of the Plagiostomi and is, in *Ceratodus*, definitely premandibular in position.

The velum of *Amphioxus*, like that of *Petromyzon*, is not terminal in position.

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REVIEWS

The Mammal-Like Reptiles of South Africa and the Origin of Mammals. By Robert Broom, D.Sc., F.R.S. (London: Witherby.) 1932. Pp. 375. Text-figures 111. Price 25*s*.

After a prolonged and uphill fight triumph has come to Dr Robert Broom. A medical graduate of the University of Glasgow, always dependent on his profession for a living, he has succeeded in establishing for himself a place amongst the great discoverers of his time. He went to Australia in 1893 but we suspect it was the fauna as much as opportunities for practice that tempted him to that continent. No sooner was he there than we find him immersed in problems which had attracted him in Glasgow—the evolutionary changes of the palato-pterygoid-quadrate arch—which from first to last, have never been lost sight of. And not much encouragement was given him to pursue his anatomical studies, as the following sentence serves to illustrate.

"From an examination of the early chondrocranium of the marsupial in 1896 I came to the conclusion that the alisphenoid is really a development of the palato-pterygoid-quadrate arch; and sent off to London in 1898 a short paper expressing this view. The pundits, however, evidently considered the paper too heretical and declined it."

In 1897 he resolved to seek his fortune in South Africa and again we suspect he was tempted to that part of the world not by what it had to give to medical men but by the opportunities it afforded for one who had made up his mind to resolve the problems of cranial morphology. For by 1897, thanks to the labours of Owen, Cope, Seeley and many others it was known that the Karroo formations of South Africa were a vast and rich store-house of the fossil remains of a puzzling reptilian fauna—one showing unexpected resemblances to mammals.

"Our fossil beds," says Dr Broom in 1932, for 35 years of residence had given him the South African point of view, "cover an area of 200,000 square miles, in almost any area of which fossils may be found.... I estimate that there are lying to-day exposed to view the fossil remains of five animals to every square mile. In some areas there are 100....if the wind-blown sand and dust could be removed there would be 200 million fossil animals exposed to view."

The beds, if superimposed, would have a thickness of 15,000 feet; they began to be laid down in the Permian and continued to the Upper Triassic—a period estimated at fifty million years. Reptilian fauna succeeded reptilian fauna, but as time went on mammal-like features became more emphasised so that by the end of the Triassic, one form at least—the Ictidosaurian—was assuming monotreme-like features. Altogether in the Karroo deposits Dr Broom believes there are preserved fossil remains of "millions of species." Of these millions only 350 species have so far been described; of the millions and millions of skulls only 1200 have been minutely examined. For thirty years Dr Broom has been discovering, examining and making this wonderful fauna known to the world; he has become the accepted interpreter of this great extinct kingdom of reptilian life.

From time to time he has published papers, now no longer refused by the "pundits of London." In the present book he gives a list of 117 published papers from his own pen—most of them relating to the Karroo fauna. Manifestly as his material and experience grew, his interpretations and inferences altered so that it became a matter of importance both for the rescue of his own life's work and for the benefit of all who labour at the history of living things, that his harvest should be systematised and presented to the world of science as a connected whole. This has now been done—thanks to the Carnegie Corporation of New York and the Research Board, Union of South Africa. Those who administer the estate of Andrew Carnegie never did a better act to a deserving labourer in the fields of science or for the advancement of our knowledge of the

palaeontological procession of types than when they resolved to give Dr Broom the means for publishing his labours in a befitting form.

Under these circumstances Dr Broom has published a descriptive and illustrated account of representative types of the mammal-like reptiles of South Africa—Dinocephalians, Therocephalians, Gorgonopsidians, Anomodonts and Cynodonts. Comparative anatomists have thus at their disposal the known facts relating to the evolving types which made their appearance and disappearance on the South African stage during the "age of reptiles." Dr Broom has deliberately chosen to give exact reconstructional drawings of the various types rather than actual photographs or drawings of the imperfect or distorted originals—a preference with which the reviewer is wholly in sympathy. Perhaps the more perfect way is to give both photograph and drawing but if we are to have but one then certainly it is an exact reproduction of the author's reconstruction which is to be preferred—such representation being given from defined points of view—profile, vertex, base, occiput, etc. With each drawing a scale should be introduced—so that students may realize the degree of magnification or reduction. Nowhere in his excellent drawings does Dr Broom give a scale.

As a faithful and sufficient record of fact, this handsomely produced volume—*The Mammal-Like Reptiles of South Africa*—is all that can be desired. As a recorder of fact Dr Broom is at his best; as a philosopher, seeking to interpret the forces which bring about evolutionary changes, he lays himself open to criticism. He comes to the conclusion that the forces of evolution are exhausted.

"In fact," he writes, "one feels driven to the conclusion that the further evolution of the mammals is limited to secondary modifications of existing types.... But whatever changes there may be in the future it seems quite impossible that mammals can ever again originate from reptiles or amphibians, or men from apes."

In every age there have been men who proclaimed that their particular branch of knowledge had reached its full maturity—which every succeeding generation has proved to be a false prophecy. The reviewer has no doubt if a "brainy" Cynodont had surveyed his compeers that he, too, would have failed to see the future possibilities of the forms around him; very likely when the decaying mandibular articulation in the Ictidosaurians was brought to his notice; he would have seen, not the birth of a new mechanism of hearing, but a sure sign of a coming catastrophe. Those living some ten millions of years hence will know how far Dr Broom has been a true prophet. The late Dr William Bateson failed to produce a new species by artificial breeding and was inclined to think that it could not be done. And yet the world is full of evidence that it has been done and there is no fact—except our ignorance—which leads us to believe that nature is less fertile to-day than she has been heretofore. Life has never stood still and we cannot conceive any method which will prevent evolutionary changes from proceeding—except man's wilful interference or destructive recklessness.

Dr Broom lays before the reader his opinions of the various accepted modes in which new species have been evolved amongst the mammal-like reptiles of South Africa.

"What has produced the different species?" he asks. "It seems to me that natural selection has nothing to do with the production of new species. It doubtless weeds out the less satisfactory types but there is something else that produced the new. Lamarckism also seems quite inadequate....South Africa will yet give a far fuller account of the evolution of the mammal-like reptiles, and clearly show every step that has led to the warm-blooded mammals. We will then know whether the appearance on the earth of mammals has been the result of blind chance or whether it has been due to the guidance of some intelligence."

Sir James Jeans has thrown out the suggestion that we may regard the universe as the expression of a great thought. We should be equally instructed if a psychologist were to proclaim that a thought was the expression of a great universe. The suggestion of using "intelligence" as a theory to explain evolution leaves us with the still greater

task of explaining the origin and nature of intelligence. Fortunately on another page Dr Broom gives us a more precise indication of what he implies by calling in intelligence to explain the origin of species. "It would almost seem," he writes, "as if in each animal there is some power which can act on the chromosomes, and give rise to changes to meet the new requirements of the type. Whatever the power be, it never seeks to take into consideration anything but the immediate needs, and in nearly every case the specialization results in ultimate extinction." In another passage Dr Broom exclaims: "We almost seem driven to assume that there is some controlling power which modifies the animal according to its needs and that the changes are inherited." Is this "controlling power" another term for "intelligence"?

Although Dr Broom rejects Lamarckism as a mode of evolution yet if he had not stated so categorically one would have been compelled, from the evidence of his statements, to regard him as a devout follower of Lamarck. "Most steps," he says, "seem to have been taken to meet some immediate need. A change of diet or environment resulted in a change of habit and the change in habit led to a change in structure." Or again, "The changes in the pelvis have like those in the front limb been manifestly due to changes in habit." The evolution of the new joint for the lower jaw and the consequent utilisation of the bones of the old joint by the ear, he also thinks "may have been due to some change in diet." Numerous other phrases used by Dr Broom make it difficult to distinguish between his doctrine of evolution and that of Lamarck.

The evidence produced by Dr Broom leaves the student in no doubt that towards the end of the Triassic period certain of the mammal-like reptiles of South Africa were assuming monotreme-like features in their skulls, limb girdles and limbs. The matter which we should like enlightenment on relates to the structure of their thorax. Diaphragmatic respiration is the hall-mark of the mammal. Did any of the South African mammal-like reptiles have ribs which were shaped and articulated as in monotremes? In all examples and illustrations known to the reviewer the rib series is as in reptiles and it is difficult to conceive how a mammalian diaphragm could be evolved from the form it assumes in every reptilian form known to us. It is difficult to believe, also, that at so late or high a stage of evolution, as that represented by the mammal-like reptiles of South Africa, it would be possible for the old articular bones of the lower jaw to pass into the service of the bones of hearing. There is still much evidence to be gathered before the mammalian ancestry can be traced to the reptiles of South Africa. There is still much to be said for Huxley's conception of an amphibian origin for mammals. A.K.

The Invertebrata—A Manual for the use of Students. By L. A. Borradaile, L. E. S. Eastham, F. A. Potts and J. T. Saunders. (Cambridge University Press.) 1932. Pp. xix + 645. 458 figs. Price 25*s.* net.

This advanced text-book on the Invertebrata by four well-known Zoologists is very welcome, as it is many years since a book of this range and quality has been published in England. It is designed for the use of honours students and gives, though necessarily in a condensed form, a remarkably searching and at the same time readable account of the structure and classification of the various divisions and subdivisions in which this immense assemblage of animals is graded. The method of presentment is somewhat similar to that adopted by Sir Ray Lancaster in the *Oxford Natural History*; each subkingdom and phylum is introduced by a general account of the more salient and characteristic structural features of its members; this is followed in each case by shorter descriptions of the peculiarities of structure exhibited by the lesser groups within the phylum, to which finally are added a few notes on specially selected genera.

The information given is primarily morphological, with emphasis laid on those

characters that indicate community of origin or suggest probable trends of evolutionary change, the whole marshalled with the object of establishing a natural system of classification. At the same time, although the keynote of the book is systematic morphology, the authors have been careful not to lose sight of the fact that their subject has also a physiological aspect, and throughout the work, as opportunity arises, introduce much interesting matter relative to the action of organs, the reaction of the animal to its environment, and the vicissitudes of its life history. This broadly physiological note is especially evident in the chapter on the Protozoa, but may be recognised as an undercurrent throughout the book.

After a short introduction indicating the scope of the book and the reasons for dividing the whole animal kingdom into three subkingdoms—represented by the Protozoa, Sponges, and Metazoa—in which the gulf between the Protozoa and Metazoa is so deep as to suggest that both arose independently from a primeval living stock common to them and the plants, the authors enter upon the body of the work with a detailed description of the structural peculiarities of these three subkingdoms, devoting a long chapter of more than 100 pages to the Protozoa, a few pages to the Sponges, and the rest of the book to the Metazoa.

In treating of the Metazoa most attention is given to the larger and more important phyla whose relationships to one another are fairly established. Following the general consensus of opinion a primary distinction is drawn between the two-layered Coelenterata and the rest of the subkingdom in which a third (mesodermal) layer has been evolved; within this triploblastic remainder two associations, the result of separate lines of evolution, are recognised, based mainly on the mode of origin of the mesoderm and on the type of cleavage of the ovum. The one association includes the Annelida, Arthropoda and Mollusca, the other the Echinodermata and Chordata (leading to the Vertebrata and Man); in accordance with the theory put forward by Prof. MacBride.

The true relationships of the smaller phyla are still very obscure; and, perhaps wisely, no dogmatic statements are made about them; it is, however, suggested that probably the Platyhelminths, the Rotifers and the Polyzoa have affinities with the Annelids, and the Brachiopoda and Chaetognatha with the Echinoderm-Chordate stock. With regard to the more detailed classification within the several phyla, the student is warned to keep an open mind and not to assume that the scheme set forth is in any way final.

A book covering so large a field and containing such a mass of condensed technical information does not lend itself to quotation, though it contains many passages of interest to others than those for whom it is primarily written; such for instance are the pages on the ancient history of the Insecta (p. 404), in which is recorded the simultaneous advent upon the earth of the higher insects and the flowering plants; or the account of the curious association of the reproductive activities of certain sea worms with the phases of the moon (p. 246), or the behaviour of the Protozoa in relation to environmental stimula (p. 33). A word must be said of the illustrations which, though rather small, are numerous, clear and well selected; most of them are line drawings. There is also a useful comparative table of the somites and limbs of Arthropoda.

No bibliography is included. This is perhaps a pity, for although no doubt the study of original sources is not strictly required of the student, a carefully selected list of leading original papers would undoubtedly be of service to him after his student days as an introduction to the study of literature: an absolutely necessary part of any research upon which he may embark.

Outline of Comparative Embryology. By Prof. AUTE RICHARDS. (New York: John Wiley and Sons, Ltd.; London: Chapman and Hall, Ltd.) 1931. Pp. xvi + 444. 30*s.* net.

The first part of this book deals with those embryological processes which are common to most of the higher animal forms, and presents in assimilable form the accepted view on cleavage, germ layer formation, the egg and embryonic membranes, including the placenta. The consideration of various invertebrate forms is based largely on the work of Korschelt and Heider. The second part of the book deals with modern problems in embryology and presents the modern aspects of asexual reproduction, parthenogenesis and polyembryony with clarity. The historical aspect is well maintained and the recent expansion of experimental embryology is admirably incorporated.

Cicatrisation et régénération. Par Prof. JACQUES MILLOT. (Collection Armand Colin: Section de biologie, No. 141.) (Paris: Armand Colin.) 1931. Pp. 204. 10.50 francs.

A brief account of the present views of wound healing and regeneration of parts in the animal kingdom with special regard to the more recent experimental methods in the lower animal forms. The physiological factors involved are analysed and there is an interesting account of the corresponding compensatory mechanism in plants.

Grundriss der allgemeinen Zoologie. Von Prof. Dr ALFRED KÜHN. Vierte, verbesserte und vermehrte Auflage. (Leipzig: Georg Thieme.) 1931. Pp. viii + 264. 13.20 gold marks.

The *Grundriss* of Prof. Kühn, now in its fourth edition, has become a standard text-book of Zoology for medical students. It is characteristic of the modern trend in morphology that considerable space is devoted to animal parasites and to heredity. This book can be recommended to English students of Zoology as a rapid means of acquiring familiarity with the German language, for the copious illustrations are simple and clearly labelled.

Zoologie biologique. Par Prof. ÉTIENNE RABAUD. Fascicule 1: Morphologie générale et système nerveux. (Paris: Gauthier-Villars et Cie.) 1932. Pp. xxi + 223. 45 francs.

This is the first of three volumes which deal respectively with general morphology in relation to the nervous system, nutrition and reproduction. The first volume approaches the problem of general morphology by describing the fundamental structure of all animal forms in terms of the integument, organs of locomotion and the body cavities.

The relation of the organism to the environment is approached by the study of the peripheral nervous system, with special attention to the nerve endings of ordinary and special sensation. The author has succeeded in introducing many of the conceptions of modern physiology into morphology.

Vorlesungen über vergleichende Anatomie. Von Prof. Otto Bütschli. Lieferung 5: Leibeshöhle. Ueberarbeitet und herausgegeben von C. Hamburger. (Berlin: Julius Springer.) 1931. Pp. iv + 381–490. 16.80 gold marks.

This section of Bütschli's text-book of Comparative Anatomy, commenced by Blochmann and completed by Hamburger of Heidelberg, deals with the body cavities. One half of the text is devoted to the invertebrates and one half to the chordates. The development of the coelom, mesenteries, pericardio-peritoneal septum and diaphragm is traced with the aid of simple diagrams. The abdominal pore is traced through cyclostomes, Fishes and Birds. The facts of embryology are incorporated in the text with clarity. The absence of theorising and the excellence of the diagrams are to be commended.

Comparative Anatomy of Vertebrates: an Outline of a Laboratory Course. By Herbert W. Rand. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press.) 1931. Pp. 78. 6*s.* net.

A brief laboratory handbook based on the familiar outline of G. H. Parker, devoted to the descriptive anatomy of a typical Amphibian, Reptile, and Mammal. To what extent such outlines instil enthusiasm for truth, critical analysis and logical deduction is doubtful.

Entwicklungsphysiologie der Tiere. Von Dr Paul Weiss. (Wissenschaftliche Forschungsberichte, Naturwissenschaftliche Reihe, herausgegeben von Dr Raphael Ed. Liesegang, Band 22.) (Dresden und Leipzig: Theodor Steinkopff.) 1930. Pp. xi + 138. 11 gold marks.

This presentation of border-line problems has no counterpart in the English language. Prof. Weiss has succeeded in bringing together under the title of Developmental Physiology a survey of the present fluidity imposed by Experimental Embryology on the older and somewhat effete Morphology. This is the best presentation of the post-war change in outlook in Biology.

The book deals in particular with the balanced growth of the embryo, organ formation and the rôle of Spemann's "organisators." Growth is analysed in terms of cellular proliferation and cellular differentiation and the characteristic features of growth *in vitro* and *in vivo* are compared. Metamorphoses and regeneration lead up to a final chapter dealing with recent theories in Biology.

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